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A Study of the Serological Races of the
Flexner Group of Dysentery Bacilli



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MEDICAL RESEARCH COMMITTEE

**A Study of the Serological Races of the
Flexner Group of Dysentery Bacilli**

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INTRODUCTION

READY means of identifying the slightly varying races of bacilli belonging to the 'Flexner' group of organisms has been one of the chief bacteriological needs during the war as an aid to the proper study and control of dysentery epidemics caused by infections of this group. At the outbreak of war only a few of the existing and already recognized strains of Flexner organisms were available in this country for the production of diagnostic sera, and these were not well representative of the whole group in their range. In 1907, W. Kruse in Germany had classified and described many strains collected from outbreaks of dysentery at various places, but no specimens of his identified strains appear to have been available in this country or in France or America at the outbreak of war, and they have naturally been unavailable since.

In their previous Annual Reports the Committee have given brief accounts in successive years of the efforts which have been made by bacteriologists within and without the Navy or Army Medical Services, to secure the easier detection of Flexner dysentery by proper recognition and classification of the Flexner variants. In 1915, very large numbers of dysentery patients and convalescents were received in this country from the Mediterranean areas, and in a great majority of these no diagnosis had been made of the determining cause of the dysenteric symptoms. The Committee were able to assist the War Office by arranging for skilled bacteriological work at various chosen centres of work, and the immediate results of much of this work have been published already in earlier numbers of the present series of Reports and elsewhere. From 1916 onwards, outbreaks of dysentery arose from time to time in France as men formerly engaged in the East were increasingly drafted to the armies there. Both in the laboratories at home and in those overseas, it became increasingly important for the diagnosis of dysentery cases and for the detection of 'carriers' that diagnostic sera should be available for the identification of all the possible races of the Flexner bacilli. It was found everywhere that organisms assignable by cultural and chemical characteristics to the Flexner group failed to respond in the final test by agglutination with any of the diagnostic sera then obtainable. Many of these 'aberrant' races were collected by various observers for closer study, and by interchange between different centres of work some progress was made. It became clear, however, that a more rapid understanding could be reached of the relationships of these aberrant forms by more intensive study of a centralized collection.

In consultation with the Army Medical Department, accordingly, the Committee arranged early in 1917 that Dr. A. C. Inman (Hon. Captain, R.A.M.C.), Pathologist to the Brompton Hospital, at that time working on their behalf, should devote himself to the collection and classification of aberrant types isolated from dysentery cases or convalescents at various centres. Captain Inman had worked for some months in the first place at the Mont d'Or Military Hospital, Bournemouth, one of the special centres for dysentery work. On behalf of the Committee he visited the bacteriologists in charge of other centres of dysentery work, and by their courtesy was able to gain their experience with aberrant 'Flexner' types and to collect examples of these for later comparison. At the same time Dr. H. S. Gettings had been working for the Committee at St. Mary's Hospital, with the active encouragement of the Board of Control, upon the same problem as it had presented itself in the outbreaks of Flexner dysentery which had long been a matter of concern in various asylums. In September, 1917, the number of aberrant types reported in the armies in France had led to increasing uncertainties in work, and Colonel G. S. Buchanan, C.B., of the Local Government Board, and Professor F. W. Andrewes, F.R.S. (then Major, R.A.M.C., T.F.), of St. Bartholomew's Hospital, proceeded for a short visit to France at the request of the Director-General, A.M.S., to consider and report on the problems presented. After their return it was arranged that Captain Inman should be further freed from routine work at Bournemouth and should give his whole time to the study of Flexner races in the Pathological Department, St. Bartholomew's Hospital, where a room was kindly put at his disposal for this purpose. Here many cultures

of aberrant Flexner types were sent by direction of the War Office from laboratories abroad and at home, and the inquiry of which the results are now given was conducted by Major Andrewes and Captain Inman in collaboration. The agglutination technique employed here was in the main that recommended by Professor Dreyer, and arrangements were made at the Oxford Standards Laboratory, maintained by the Committee for the War Office under Professor Dreyer's general direction, to prepare standard cultures and agglutinable sera in respect of particular Flexner types when recognized and grouped at St. Bartholomew's. In May, 1918, the War Office Dysentery Committee was constituted, and all the work being done upon dysentery, whether bacillary or amoebic, was thereafter brought under the review of that Committee with a view to its application in administrative practice. In August, 1918, it appeared desirable that Captain Inman should proceed to France, and by the examination of cases and of bacillary types isolated in the laboratories there, test the progress already made. Taking advantage of arrangements effected by Colonel S. L. Cummins, C.B., C.M.G., Adviser in Pathology, A.M.S., who gave every possible assistance to the inquiry, he was able to work for a time with Major Marrian Perry, R.A.M.C., in charge of the laboratory of No. 14 Stationary Hospital, Wimereux, a chief collecting centre for dysentery cases, where Major Perry, with Captain H. W. Perkins, R.A.M.C., not only placed the fullest laboratory facilities at Captain Inman's disposal, but actively co-operated with him in the carrying out of the investigations which he had in hand. The Committee were also able to arrange, by permission of the American military authorities, for Captain Inman to work for some weeks at the American Central Laboratories at Dijon, where, by the courtesy of Colonel J. F. Siler, Director of the Laboratories, he had the advantage of gaining fresh experience of types isolated in the American Forces.

The present Report gives the conclusions at which Professor Andrewes and Dr. Inman have been able to arrive, and codifies the classification of the races of the Flexner group, as determined by serological study, which they now put forward. As the authors observe, 'the opportunity presented by the recent war for a comprehensive study of the dysentery group has been unrivalled. . . . The war seems to have brought together examples from all parts of the world, and it has been possible to collect a series of representative strains on a scale which has never been attained before and is unlikely to occur again'. The Committee think it will be generally admitted that the authors have fully availed themselves of the opportunity so presented, and have made an important contribution to the ease and accuracy of all future work in this direction.

The Committee would venture again here to acknowledge their indebtedness for the co-operation which the Directors-General both of the Navy and Army Medical Services have made it easy to establish between the work done in those Services and the parallel work done by civilian investigators elsewhere. In particular the Committee would join with the authors of this Report in expressing their thanks to the numerous observers who have contributed to this investigation by the supply of bacillary cultures of previously unrecognized type isolated in the course of their work. Without these contributions, the work, however skilfully done, would have had little general value. Its essence lies in the degree to which it covers in its classification the widest range of organisms within the whole group. Special acknowledgement should be made here of the services rendered by Captain William Fletcher, R.A.M.C., Bacteriologist to the Southampton War Hospital, who gave invaluable assistance throughout the inquiry by supplying material from his laboratory, and in other ways.

The authors mention in their Report that their results are in general agreement with those independently reached by Dr. H. S. Gettings, to whom reference has already been made. Dr. Gettings' untimely death in 1918 cut his work short. It is greatly to be hoped that the results now reached may have special value in the direction at which he had long aimed, namely, the better control of asylum dysentery, which in this country and in time of peace offers perhaps the largest practical problem presented by this group of infective organisms.

MEDICAL RESEARCH COMMITTEE,
15 Buckingham Street,
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28 October, 1919.

A STUDY OF THE SEROLOGICAL RACES OF THE FLEXNER GROUP OF DYSENTERY BACILLI

By F. W. ANDREWES AND A. C. INMAN.

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I. INTRODUCTION.

THE present essay is limited in its scope. It makes no attempt to deal fully with the wide question of what organisms are to be included in the circle of true dysentery bacilli: its aim is to define the limits of a single species, commonly known as Flexner's bacillus, and to discuss the serological races which exist within the limits of that species.

Two organisms are by universal consent credited with a causal relationship with epidemic dysentery—Shiga's bacillus and Flexner's bacillus. Apart from these, similar claims have been made, especially during the recent war and on the Eastern fronts, on behalf of a number of related species, all belonging to the same generic group. The evidence has been sometimes good, sometimes doubtful, but, so far as a relation to widespread epidemic dysentery is concerned, by no means of equal value to that which can be adduced in the case of the two first-named organisms.

Of these two, Shiga's bacillus is the more dangerous: it exhibits a higher degree of toxicity and sets up a more severe form of dysentery with a higher rate of mortality. It appears a more specialized type, with less affinity than Flexner's bacillus with other species of the genus. It is also a more homogeneous type, not presenting any well-marked serological races. No serious difficulties have arisen during the war in the identification of Shiga's bacillus.

It is otherwise with Flexner's bacillus. Not only is this organism more closely related to adjacent species, so that its specific limits are not easy to define, but within these limits it presents a number of serological races, failure to recognize which has led to much uncertainty in diagnosis. It has been a common experience in the examination of cases of dysentery to isolate bacilli conforming culturally to the Flexner type, but failing to agglutinate with the Flexner and Y sera available for serological confirmation. In the sero-diagnosis of dysentery the same difficulty has been encountered: the range of agglutinable emulsions provided for this purpose has apparently been too limited, for many cases of undoubted bacillary dysentery have yielded negative results.

Under these circumstances it seemed a matter of some urgency that a closer serological study of the races of Flexner's bacillus should be undertaken. By arrangement with the military authorities and by the kindness of friends, a large number of strains were collected at St. Bartholomew's Hospital, and this report deals with the conclusions reached from their examination.

Historical Summary.

In its epidemic form, dysentery is a true specific fever, and in such epidemics the amoebae which had been shown to be associated with the endemic dysentery of the tropics were not demonstrable. The first to discover a bacillary cause for epidemic dysentery was Shiga, who, in Japan, demonstrated in the stools a special type of bacillus which was agglutinated by the serum of the patient, and which he named *B. dysenteriae*. Shiga's work was published in

1898, and his discovery was soon confirmed by Kruse, who found the same bacillus in epidemic dysentery in Germany. Kruse corrected certain inaccuracies in Shiga's description, and the organism is sometimes called the 'Shiga-Kruse' bacillus. Confirmation came also from America and from the Philippine Islands, where the American workers, chief of whom was Flexner, found in the local dysenteries bacilli which appeared to conform to the Shiga type (1900).

A new complication now arose. Kruse had been unable to demonstrate Shiga's bacillus in the asylum dysenteries in Germany, but had found bacilli evidently closely allied to it. On the assumption that true bacillary dysentery must be due to Shiga's bacillus, he called these asylum dysenteries 'pseudo-dysentery', and he has continued to speak of the organisms found in them as 'pseudo-dysentery bacilli'. Many of the strains isolated from dysentery cases by Flexner and his colleagues in the Philippines and in the United States were similarly found to differ from the classical type of Shiga, and ultimately proved identical with Kruse's pseudo-dysentery bacilli. The work of Martini and Lentz, published in 1902, finally established the clear serological differences separating Shiga's bacillus, on the one hand, from the mannite-fermenting strains (Flexner and pseudo-dysentery) on the other. It was not, however, at that time clear whether these forms were varieties of a single species or specifically distinct. It was nevertheless recognized that definite serological differences existed amongst the various 'Flexner' strains themselves, and some of these received names, such as the 'Strong' type. In 1903 Hiss and Russell described a form which they called the 'Y bacillus', isolated from a case in the United States.

In 1904 Hiss published a classification of the dysentery bacilli, based largely on sugar reactions, but also on serological data. He considered that there were four races:

1. *Shiga's bacillus*, which is unable to ferment mannite.
2. *Flexner's bacillus*, which ferments mannite and maltose.
3. *Strong's bacillus*, which ferments mannite, maltose, and saccharose.
4. *The Y bacillus*, which ferments mannite but not maltose.

This terminology has been in more or less general use up to the present time, and is that found in most text-books, although it has been known for several years that the fermentative powers of this group of organisms are too liable to vary to afford a secure basis of classification.

At the present day, Shiga's bacillus is conceded the rank of a distinct species. It is culturally distinguishable by its inability to ferment mannite and to form indol, while its toxicity is notably higher than that of the mannite-fermenting group. It is sharply defined by its serological characters. The antibodies which it evokes in man and animals possess a high degree of specificity, reacting but slightly, or not at all, with the mannite-fermenting group of bacilli, while the antibodies formed in response to the latter react with Shiga's bacillus to an equally negligible degree.

Turning now to the Flexner group of organisms, we find in recent

years a general consensus of opinion that their sugar reactions do not afford a reliable means of dividing them into sub-groups. Already in 1907 Kruse had found the fermentation of maltose and saccharose variable and uncorrelated with serological properties. A number of workers during the recent war have expressed similar views, amongst them C. J. Martin, Gettings, Murray, and Dudgeon—indeed it would now be difficult to find any responsible bacteriologist to defend a sub-grouping based on the power of splitting up maltose and saccharose. The reactions with lactose, mannite, and dulcitol are on a different footing, since they exhibit a fairly well-marked correlation with serological properties.

Although it is on serological properties that classification must ultimately be based, it is a remarkable thing that the majority of workers, before the recent war, failed to produce any trustworthy classification of the Flexner group on these lines. Agglutination, bacteriolysis, and complement fixation were tried, but the attempts were for the most part abandoned. A noteworthy exception is to be found in the work of Kruse and his collaborators at Bonn, who studied the subject for several years, testing all the strains of 'pseudo-dysentery' they could obtain. In addition to agglutination and agglutinogenic power, they made extensive use of the absorption test, and in 1907 an important paper was published in which, after separating off Shiga's bacillus, they described the mannite-fermenting forms as divisible into a number of serological races which were lettered A, B, C, &c. The first five races were clearly defined, but F, G, and H were left somewhat indeterminate. Race A was the local form prevalent at the Bonn lunatic asylum, B represented the Flexner strains of American origin; of race C only two examples were found, but D was responsible for an extensive local outbreak, and was regarded by Kruse as possibly identical with the Y bacillus of Hiss and Russell, although he possessed no authentic strain of the latter with which to compare it. Race E consisted of lactose fermenters, which were unhesitatingly included with the pseudo-dysentery bacilli. This work marks an important stage in our knowledge, since it was the first serious attempt to employ modern serological methods in the grouping of the mannite-fermenting dysentery bacilli. Kruse's classification gained wide acceptance in Germany, and was confirmed by other workers there. In other countries it was less known, and at the outbreak of the war no authentic strains of Kruse's races were available in this country, in France, or in America.

Our position indeed was this. We had no difficulty in recognizing Shiga's bacillus, but we were not able to define the Flexner group with any certainty, and as regards its constituent races we spoke only of 'Flexner', 'Y', and perhaps 'Strong'. We did not know which of the strains current in the different laboratories was the authentic Y, for the term was loosely used for almost any strain which did not agglutinate with a 'Flexner' serum. When a strain fermented saccharose we surmised it to be a 'Strong', but the so-called 'Strong's' preserved in many laboratories had no further authenticity, and indeed appear to belong to a species different from Flexner's bacillus.

The opportunity presented by the recent war for a comprehensive study of the dysentery group has been unrivalled. Most of the work which had hitherto been done had been on strains from localized outbreaks, or limited areas, in which only a few types had been represented. But the war seems to have brought together examples from all parts of the world, and it has been possible to collect a series of representative strains on a scale which has never been attained before and is unlikely to occur again. Of this opportunity we have endeavoured to avail ourselves.

II. DEFINITION OF THE FLEXNER GROUP OF DYSENTERY BACILLI.

It is clearly necessary, as a preliminary to any study of a group, to frame some definition of its limits. We must therefore discuss this subject first and state our reasons for a limitation which some may regard as arbitrary.

The bacilli credited with the causation of dysentery all belong to a natural genus which presents the following characters. They are short rods, destitute of flagella and non-motile, forming no spores, not liquefying gelatin, negative to Gram's stain, and fermenting glucose and sometimes other sugars and alcohols without the formation of gas.

The properties which may be used for the sub-division of the genus into species are as follows: (1) sugar reactions and other biological properties, such as the formation of indol, alkali formation, &c.; (2) pathogenicity; (3) serological characters; (4) reaction to acid agglutination.

1. Fermentation Reactions.

Experience has limited the fermentable substances of real service in the classification of the dysentery group to four, viz. glucose, mannite, lactose and dulcitol. All the members of the group form acid with *glucose*.

By general consent, the division of the group into two sections, according to whether *mannite* is, or is not, fermented, is a valid one. Shiga's bacillus does not ferment it, nor does the indol-forming species described by Schmitz (apparently identical with the type described by one of us as '*B. ambiguus*'). Two strains which we have received as 'Strong' types also failed to ferment mannite,¹ and on other grounds were clearly separable from the Flexner group.

The mannite-fermenting section of the dysentery group is again divided into two sections by the reaction with *lactose*. The division is not quite sharp. Even the species regarded as non-lactose-fermenters often attack this sugar in very slight degree. Winter claims that Shiga's bacillus does so, but the proof demands elaborate chemical analysis. If one employs a medium just neutral in reaction and relatively free from buffer substances (e. g. ovomucin), a delicate

¹ It may not be superfluous to remark that care should be exercised to use pure mannite, as certain samples in the market are untrustworthy. A simple test is offered by the polarimeter: pure mannite deflects polarized light very slightly to the left.

indicator, such as that of Andrade, often reveals slight acidity with lactose after twenty-four hours in the case of both Shiga's and Flexner's bacillus, more especially with strains which have been long under cultivation. The amount of acid formed is slight, and soon overborne by the alkali produced by protein cleavage, so that the phenomenon is transient. In the case of the true lactose fermenters the acidity is permanent, but even here there is commonly a certain hesitation about the attack on this sugar. Some members of the group form acid in twenty-four or forty-eight hours, but more commonly the acid reaction with lactose is delayed for several days or even for two or three weeks, so that there is a liability for such types to be wrongly diagnosed.

But if allowance is made for the facts just mentioned, and transient initial acidity is disregarded, it remains true that the reaction with lactose effects a useful division of the mannite fermenters into two groups. The reality of the distinction is strongly supported by the lack of serological conformity between the two sections. Flexner's bacillus, by general consent, belongs to the group which does not ferment lactose, but it is not the only species in this section. The species which ferment lactose, early or late, are also probably several in number: some of them are pathogenic for animals, and are claimed as causal agents in bacillary dysentery.

The reaction with *dulcitate* effects a further differentiation. Certain of the lactose fermenters attack *dulcitate* also, but the chief value of the *dulcitate* reaction lies in distinguishing between Flexner's bacillus and the species which one of us has described as '*B. alkalescens*'. Flexner's bacillus does not ferment *dulcitate*, but *B. alkalescens* does so. The latter species is also distinguished by the intensity with which it produces alkali in milk and sugar-free proteid media, and by its lack of toxicity.

Reactions with *maltose* and *saccharose* are here disregarded, though reliance was formerly placed on them. They have proved too variable for use, being frequently lost or gained by individual strains under cultivation. The reaction with *saccharose* may exceptionally be seen in various races of Flexner's bacillus, and may be late in appearing. It has been regarded as a criterion of the so-called 'Strong' type, but it seems now impossible to decide what this type really was.

The *indol* reaction is of service in discriminating between Shiga's bacillus and the type described by Schmitz. In the mannite-fermenting group it is too variable to be of any use in classification.

It will be seen from the foregoing remarks that by the use of four sugar tests and of the *indol* reaction, a considerable amount of grouping can apparently be effected in the genus under consideration. The validity of such grouping must, however, be judged by its correspondence with that brought to light by other tests.

2. Pathogenicity.

The pathogenic powers of this group of organisms upon animals are, it must be confessed, of less service in their classification than might have been expected. It is difficult to reproduce, in laboratory animals, the lesions of human dysentery, though in certain cases

this has been accomplished. Nevertheless a number of species in the group are definitely pathogenic for animals, and not infrequently display a selective toxic action upon the intestinal mucosa.

One species, Shiga's bacillus, stands apart from the rest in the high average degree of toxicity which it exhibits, and the particular effect produced by its toxin upon the mucosa of the colon. At the other end of the scale, the dulcitate-fermenting *B. alkalescens* appears devoid of pathogenic power. Between these two extremes lie the other named species of the group, and probably some unnamed ones also. Different observers vary in their accounts of Schmitz's bacillus: most workers in France have reported it devoid of toxicity; others on the Eastern fronts have found it at times as toxic as Shiga's bacillus, and it is not yet clear whether the types under consideration are identical. Flexner's bacillus, intravenously injected, is very definitely pathogenic for the rabbit, and so are certain of the allied lactose fermenters and other forms which have as yet received no name; in such cases reddening and inflammation of the intestinal mucosa may be seen, though less evidently limited to the colon than in the case of Shiga's bacillus, and if the animal lives long enough it may show paralytic symptoms. But there is so much similarity between the effects of these different species that we cannot usefully employ pathogenic power as a criterion of specific difference. It is possible that there is an endotoxin, perhaps common to several members of the group, which is greatly exalted in Shiga's bacillus and of lesser potency in others.

3. Serological Characters.

The best known serological properties of this group are those relating to agglutination, but all that is known about them in other respects is in harmony with the facts revealed by agglutination. Dysentery bacilli are less easily agglutinated by immune sera than are members of the enteric group: the serum must act for a longer period, and even then the flocculation is finer and more granular. Different members of the group vary, however, in their agglutinability. Some, such as Shiga's bacillus, and, with rare exceptions, Flexner's bacillus, agglutinate with fair readiness, flocculation being nearly complete in four to six hours at 55° C. Others, such as the lactose fermenters and *B. alkalescens*, show only traces of agglutination by their immune sera after so short a period, and require 20–24 hours at 55° C. before agglutination is completed. In certain Flexner strains a somewhat similar delay in agglutination has been occasionally noted, but on the whole the difference between the early and late agglutinating species is so striking that it may almost be regarded as of specific significance.

The specific nature of the reactions with immune sera prepared from the individual species in the group is naturally judged by the degree of group-agglutination manifested, and this seems to vary according to the technique employed. Many writers have insisted on the presence of a considerable degree of coagglutination between Shiga's and Flexner's bacilli, especially in sera from the horse and goat, but also in the serum of human dysentery. Our own experience is

limited to human and rabbit sera, in which we find little evidence of such coagglutination between different species¹ (though it is marked between the different races of Flexner's bacillus). We attribute this to the use of Dreyer's agglutination technique, which, as is well established in the enteric group, reduces group agglutination to a minimum, thereby allowing specific differences to be more clearly brought out.

Our experience has been as follows. Shiga's bacillus, always well agglutinated by a Shiga serum, is practically untouched by other sera. Different strains of Shiga vary somewhat in the degree to which they are agglutinated by a given Shiga serum, but there is no evidence of the existence of any marked serological races within the limits of the species, i. e. the species is serologically homogenous.

Flexner's bacillus is not agglutinated by a Shiga serum, but it appears, from the observations of others no less than in our own experience, to be serologically a heterogenous species, in the sense that well-marked serological differences exist among its various strains. But in addition to such differences there exists also a marked tendency to group agglutination amongst the different Flexner races, suggesting that they have much in common.

Sera prepared from *B. alkalescens* and from certain lactose fermenters betray a fairly high degree of specificity for their several species. Such sera are inactive as regards Shiga's bacillus, but with Flexner's bacillus there is some slight group agglutination, more marked with certain Flexner strains than with others. Further, certain Flexner sera, if allowed to act for twenty-four hours at 55° C., produce some degree of agglutination with *B. alkalescens* and certain lactose fermenters. It would thus appear that these forms have more in common with Flexner's than with Shiga's bacillus, though very much less than that seen amongst the individual races of Flexner's bacillus.

Our experience with sera prepared from Schmitz's bacillus (or from the possibly identical *B. ambigua*) is small. Such as it is, it suggests marked heterogeneity amongst the different strains of this bacillus, and little relation with Shiga or Flexner.

On the whole, the evidence from agglutination supports the grouping suggested by the sugar reactions of this group of bacteria.

4. Acid Agglutination.

Michaelis has suggested a series of dilutions of acetic acid with caustic soda, which offer an ascending scale of hydrogen-ion concentrations. Fresh agar emulsions of bacteria in distilled water, added to the acid solutions, and incubated for two hours at 37° C., may or may not show agglutination. Typhoid and paratyphoid bacilli are agglutinated, but *B. coli* is usually not agglutinated, nor are dysentery bacilli. Michaelis shows further that if a trace of normal serum is added to the mixtures, *B. coli* is now agglutinated, while dysentery bacilli are still unaffected.

These statements of Michaelis, which, so far as dysentery bacilli are concerned, seem intended to apply only to the Shiga and Flexner

¹ See Table VII on p. 37.

types, have been more or less confirmed by other workers, notably by Hirsch, though not so fully by Eisenberg. As regards Flexner's bacillus they appear somewhat too absolute, for some old strains do agglutinate with Michaelis's solutions, though not to the same coarse degree as other species. The main facts seem correct, and one of us has applied the test to a considerable series of species belonging to the dysentery group. The results were as follows :

Shiga's bacillus never showed gross flocculation. Schmitz's bacillus always showed it. Flexner's bacillus rarely showed flocculation ; when it did, the flocculation was fine. *B. alkalescens* always showed gross flocculation, and so did many, but not all, of the lactose fermenting types. A race of bacilli isolated by Gettings from asylum dysentery, which gives the sugar reactions of Flexner's bacillus, but is serologically unconformable with that species, differed also from it in showing gross flocculation with Michaelis's solutions.

It would thus appear that in the dysentery group in its wide sense, the test of acid agglutination has considerable, though not absolute, value, in separating off Shiga's and Flexner's bacilli from other members of the group.

Conclusions.

The foregoing statements may be now summarized in tabular form, so far as concerns the principal members of the ' dysentery ' group which have been studied.

	Fermentation reactions.				In- dol.	Toxicity for rabbit.	Specific agglutination with rabbit sera.						Acid aggluti- nation.
	Glucose.	Mannite.	Lactose.	Dulcitol.			Shiga serum.	Schmitz serum.	Flexner serum in variety.	<i>B. alkalescens</i> .	Lactose fer- menters in variety.	Gettings's bacillus.	
Shiga's bacillus	+	—	—	—	—	Very high	+	—	—	—	—	—	—
Schmitz's bacillus	+	—	—	—	—	—	—	+	—	—	—	—	—
? = <i>B. ambiguus</i>	+	+	—	—	+	Variable	—	+	—	—	—	—	+
Flexner's bacillus	+	+	—	—	±	Moderate	—	—	+	—	—	—	—
<i>B. alkalescens</i>	+	+	—	+	+	Absent	—	—	—	+	—	—	+
Lactose fermenters in variety	+	+	+	±	±	Moderate	—	—	—	—	+	—	±
Gettings's bacillus	+	+	—	—	+	Moderate	—	—	—	—	—	+	+

TABLE I. REACTIONS OF VARIOUS BACILLI OF THE DYSENTERY GROUP.

It may be regarded as probable that the six forms shown in this table represent distinct species : the lactose fermenters here grouped together probably include more than one species. It will be observed that the six forms differ from one another in at least two characters, and some in three. The most doubtful is Gettings's bacillus, which differs from Flexner's only in acid agglutination and in its serological characters ; the distinction is, however, here so sharp that we doubt whether it should be included in the Flexner group proper.

The table embodies the definition which we have provisionally

adopted for Flexner's bacillus, namely, a non-motile, Gram-negative bacillus, not liquefying gelatin, not forming spores, producing acid but no gas with glucose and mannite, negative in its reactions with lactose and dulcitate, variable in its production of indol, negative or almost negative to acid agglutination by Michaelis's technique, pathogenic but not intensely toxic to the rabbit, and agglutinating with sera prepared from appropriate members of its species, but feebly or not at all with sera prepared from other species of the dysentery group. Bacilli of this type have preponderated amongst the members of the dysentery group isolated from cases of the disease in France during the late war, as they do also in asylum dysentery. In the observations which follow we limit ourselves to organisms conforming to this definition, which, if arbitrary, is at least precise.

III. SOURCES OF MATERIAL.

The greater part of the Flexner strains we have examined came from France, i. e. the dysentery was contracted on the western front. Forty-three strains isolated at the University War Hospital, Southampton, mostly from soldiers invalided from France, were sent us by the kindness of Captain W. Fletcher, R.A.M.C. Other strains were sent from base hospitals at Boulogne, Etaples, and Rouen, while some came from the Central American Laboratories at Dijon.

A series of strains, kindly furnished by Major Bowman, C.A.M.C., came from an outbreak in Italy in 1918. Four strains were isolated in Malta, and five at Salonica. Two came from Mesopotamia and two from the Dardanelles. One was from Madras, and four from Singapore, the latter by the courtesy of Dr. W. M. Scott. One strain from Nairobi was kindly sent from the Lister Institute. Ten strains had been isolated from soldiers and civilians developing the disease in England, and a few were obtained from the late Dr. Gettings—these being mostly from cases of asylum dysentery. In a number of our strains the country of origin was unknown.

We further succeeded in obtaining cultures of many of the classical strains of Flexner bacilli preserved in various laboratories. The American Museum of Natural History in New York kindly sent us examples of their types, including the original Y strain of Hiss and Russell, which was obtained from General Russell himself at Washington. Some of the types from New York were stated to have been isolated in the Philippines, including a Strong strain. The Lister Institute gave us a culture of their Hiss and Russell Y, believed to have been derived many years ago from the original culture. Dr. W. M. Scott presented the strains of Flexner and Y from the laboratory of Professor Lentz in Berlin, obtained shortly before the war. From Oxford we had the Flexner and Y strains in use at the Standards Laboratory—the Y having come from the Institut Pasteur. Other strains came to us from the Institut Pasteur direct. We were unable to obtain examples of Kruse's races, owing to the war.

We were enabled to compare our results with those of others who have recently been working at the subject in this country. Dr. Gettings's types were sent us from St. Mary's Hospital, and Captain E. G. D. Murray, R.A.M.C., gave us a number of the strains with

which he had been working. Amongst these were certain of the types with which Lt.-Col. Dudgeon had been working at Salonica, including his 'Gallipoli' and 'Logan' strains.

Our best thanks are due to all the numerous friends who have been so good as to supply us with cultures. It will be seen that our material has been not only extensive but of extremely diverse origin: it was naturally our aim to make it as representative as possible.

IV. METHODS OF STUDY.

As a working hypothesis we accepted Durham's conception of multiple antigenic components, with a corresponding multiplicity of agglutinins. There existed evidence of a good deal of 'group agglutination' within the limits of the Flexner species. Sera purporting to be Flexner and Y were in the market, and no others. The strains brought down by different Y sera were not always the same: the properties of the Lister Institute Y serum were by no means identical with those of the Y serum sent out by the Oxford Laboratory. We knew that a monovalent Flexner serum would agglutinate a Y bacillus to some fraction of its titre, and vice versa, and also that strains were not infrequent which were only feebly agglutinated, or not at all, by both Flexner and Y sera. Such facts suggested a complex antigenic structure in the group, and our aim was to ascertain how many antigenic components could be defined, and how far the predominance of one or another component was responsible for the apparent racial differences which existed.

It was deemed best to take a relatively small series of some twenty strains and work out their characters as thoroughly as possible, afterwards applying the results to the whole series of races at our command. It was important that we should be assured that the races taken for study belonged truly to the Flexner group. We were so fortunate as to have obtained from the University War Hospital, Southampton, a set of fourteen unselected strains from carriers, in all of whom the blood serum agglutinated the bacillus isolated from the faeces. Thus we were clear as to the authenticity of the strains as dysentery bacilli, while the fact that the patients were carriers collected from various fronts made it likely that many Flexner races would be represented, as in fact proved later to be the case. Captain W. Fletcher was kind enough to furnish a supply of human serum from each of the fourteen cases, and it was with these sera that the work of differentiation was begun. To these fourteen strains we added two obtained from Major Broughton-Alcock, R.A.M.C.—one isolated in France in 1914, said to be from the first case of dysentery on the British front—and one from Malta. Further, in order to have some basis of comparison, we added the strains kept at Oxford as types of Flexner and Y, and the Flexner and Y from Professor Lentz's laboratory in Berlin; later we added also the Hiss and Russell Y strain from the Lister Institute. Thus we began with twenty strains, finally increased to twenty-one.

Of monovalent rabbit sera, we had at the beginning a supply of the Oxford Flexner and Y sera, and a little monovalent Hiss

and Russell Y serum from the Lister Institute. It may be stated here that our later work showed that the Oxford Y was quite different from the classical Y of Hiss and Russell, which we ultimately obtained from Washington; it is an example of the race which we call *W*. The Lister Y is practically identical with the Washington strain, from which indeed it is believed to have descended.

We have employed the recognized methods of study—*agglutination* by known sera, *agglutinogenic power*, and *the absorption test*. Of the last named we have made full use, as will be seen later, elaborating the test on a quantitative basis. The evidence which it can be made to yield as to the existence of different antigenic components proved of great value. We have not employed the methods of complement fixation or of bacteriolysis. Throughout the whole work we have adopted the principle of expressing our results in graphic form, usually in percentages. Although we give our data in ordinary figures also, we believe that the graphs which are appended to this report will render the facts more readily intelligible.

Inasmuch as the exact technique employed has an appreciable effect upon the results obtained, we propose in the first place to set forth as precisely as possible the details of the methods we have used.

1. *Agglutination.*

All the important data have been obtained by the use of Dreyer's technique, with formalinized broth cultures; one of us (A. C. I.) was well versed in this method. The only departure from Dreyer's principles was that our emulsions were, from the nature of the case, unstandardized as regards agglutinability, though they were diluted to about the same opacity as the standard emulsions issued from the Oxford laboratories. The details of Dreyer's method are now so well known that there is no need to recapitulate them here. The readings were taken after four hours in a water bath at 54° C., and reduced, when needful, to 'standard' agglutination by Dreyer's reduction table. It is probable that this method gives the nearest approach to the actual titre of a serum which can at present be attained. When it is stated that the titre of a serum is 1 in 1,556, or 1 in 331, no claim to an exaggerated degree of accuracy is implied; the error may be 5 per cent., for a small slip of judgement in reading the precise grade of agglutination in the end tubes may easily make this difference in the figure obtained. The titres given are merely the figures resulting from calculation on the basis of Dreyer's table, and must be taken as the closest we can offer.

In 'typing' our extended series of strains, where it was less necessary to secure very exact figures, Dreyer's technique was departed from to the extent of using living or heated saline suspensions from young agar cultures in place of formalinized broth cultures, while a hot-air chamber at 54° C. replaced the water bath. Although the actual titres were not always the same as with the stricter technique, the percentage results were in reasonable harmony, and the method proved sufficiently accurate to enable us, in nearly all cases, to refer a strain to its proper group.

The graphic records of our agglutination results are obtained in the following manner. The bacillus to be tested is put up against a given range of sera, and the end point of agglutination determined in the case of each serum. The titres of the various sera for their homologous strains will rarely be the same, and may be widely divergent. Hence it is necessary, if we are to obtain comparable results, to express the effect of each serum upon the bacillus which is being tested as a *percentage* of the full titre upon its homologous strain. Thus if bacillus *A* is being tested with sera *B* and *C*, and we find that serum *B* agglutinates it to a dilution of 1 in 1,000, whereas it will agglutinate its own bacillus *B* to a dilution of 1 in 4,000, we can state that serum *B* agglutinates *A* to only 25 per cent. of its full titre. Similarly if serum *C* agglutinates *A* to 1 in 6,000, while it agglutinates its own bacillus *C* up to 1 in 5,000 only, we can say that serum *C* agglutinates *A* to 120 per cent. of its full titre. The difference may, and often does depend on differences of intrinsic agglutinability between the two emulsions, but where we are testing a single bacillus against a number of sera any excess or defect of intrinsic agglutinability will be the same in each case. Having ascertained the percentage degree to which each separate serum agglutinates the bacillus, we can plot out the results graphically as a series of columns side by side, the height of each column representing the percentage of its full titre to which each serum agglutinates the bacillus. We can arrange the sera in a constant order, and, if we choose, colour the columns to represent the serological groups to which the different sera belong. We thus get what may metaphorically be termed the 'spectrum' of the bacillus for a particular range of sera, and the spectra for different bacilli will be comparable, since, being expressed as percentages, the results are independent of the actual titres of the several sera.

This method of expressing results is exemplified in the figures appended to the present report. It shows at a glance the serological group to which a strain belongs, and it has the advantage, in a species such as Flexner's bacillus, which is of considerable antigenic complexity, of demonstrating the range and extent of the secondary or group-agglutination present.

2. Agglutinogenesis.

Apart from the human sera with which the research was started, we have relied on sera prepared from rabbits. We have made a large number of these, some fifteen or twenty in all. A single dose of half to one c.c. of a formalinized broth culture, given intravenously, commonly yields a serum with a titre of about 1 in 1,000 in a week, and a repetition of the dose suffices to raise it to 1 in 3,000 or more. We have not aimed at very high titres, as the values just mentioned were ample for our purposes. When the animals were ready they were bled out, and the serum, with the addition of 0.5 per cent. phenol, stored in the dark at 0° C.

Agglutinogenic power was graphically recorded in a manner similar to that employed to exhibit agglutination. The serum to be tested was put up against a suitable range of different bacillary

strains, including the homologous one. The titre for each strain, having been ascertained, was recorded as a *percentage* of the titre for the homologous strain, and a 'spectrum' for the serum plotted out on the same lines as in the case of the bacillary spectra, arranging the strains in the same constant order, and using the same colours for the different serological groups. Only, in this case, the differences of agglutinability of our unstandardized emulsions are not compensated, and affect the relative heights of the columns to some extent. The same advantages are apparent in this mode of expressing results as in the case of agglutination. Moreover, the spectra by agglutination and by agglutinogenic power can be compared, and are seen in the case of all but one of our groups to exhibit a noteworthy correspondence (Figs. II to VIII).

In the final stages of our work we endeavoured to condense this diagrammatic representation of agglutinogenic power still further. We believe that we have shown the existence of at least four antigenic components in the Flexner group, and by suitably coloured sectors of a circle, calculated from our data, it seems possible to suggest the antigenic structure of a given strain, so far as this is revealed by agglutinogenic power. The diagrams of this perhaps too ambitious attempt are appended later for what they may be worth (Fig. IX).

3. *Absorption of Agglutinin.*

We have sought to elaborate and improve the technique of absorption in more than one direction. We have used strictly quantitative methods in all cases. We have employed two or more absorbing doses in parallel series, so as to obtain a graduated absorbing effect. We have always kept an unabsorbed tube of serum along with the tubes containing the absorbing mixtures, to act as a control. The effect of the absorptions was ascertained by determining the exact titre of the serum in the control and in the absorbed sera, using Dreyer's method; it was determined in each case not only for the homologous bacillus, but for a number of strains selected as representing other serological races of Flexner's bacillus. The results were calculated as percentage reductions of the titres of the unabsorbed serum for each strain included in the test, and these were then plotted out graphically.

Serum dilution. A preliminary experiment showed that the curves so obtained were practically identical, whether the serum had been diluted 10, 30, or 100 times. We therefore used a tenfold dilution in nearly all our tests, as much lower titres could thus be measured—down, in fact, to 1 in 15: below this point values had to be reckoned as 0. Only in a few instances, where it was desired to make the absorbing dose an extremely heavy one per c.c. of serum, was a dilution of 1 in 100 employed.

Absorbing doses. We have always stated these as numbers of bacilli per c.c. of undiluted serum treated. Saline emulsions of living bacilli from twenty-four hour agar cultures were made, and a portion diluted down for counting. The counting was done with the haemocytometer under a Zeiss *F*, using weak methylene blue as the diluting fluid. If the counting cell be allowed to stand for an hour

or two, to permit the organisms to settle, this method affords fairly accurate results. Even with a tenfold dilution of serum, very heavy absorptions can be carried out. Thus four large agar plates emulsified in 20 c.c. saline will yield an emulsion of some 25,000 million bacilli per c.c., and 9 c.c. of this, added to 1 c.c. of serum, gives an absorbing dose of over 200,000 million per c.c. There is, however, little or no advantage in using such massive doses; a dose of 50,000 or at most 100,000 million per c.c. serum is sufficient to exhaust a serum so far as it can be exhausted, indeed nearly everything needful can be learned by using doses of 25,000 to 30,000 million, and with these we have usually been content as our upper limit. We have not used repeated absorptions: there seemed no need for them, and loss of accuracy in repeated manipulations is a danger to be avoided. As regards the lower limit we have once or twice pushed this down to the point at which no effect was produced. In one case a measurable absorption was effected at 30 million but none at 10 million per c.c. of serum (see Fig. X). In another case 50 million produced no effect. A very useful lower absorbing dose lies at 1,000 million per c.c. Experience led us finally to adopt two absorbing doses, 1,000 million and 20,000 or 30,000 million: these suffice for most purposes.¹

Full details of the method we employed are as follows. A fairly heavy emulsion was made from young agar cultures, and counted. Dilutions were made from this so as to contain the required number of organisms, usually 200 million and 5,000 million per c.c. Into each of three sterile centrifuge tubes one c.c. of undiluted serum was measured. To the first (control) tube 9 c.c. of saline were added; to the second, 4 c.c. saline and 5 c.c. of the 200 million emulsion, and to the third 4 c.c. saline and 5 c.c. of the 5,000 million emulsion, thus getting absorbing doses of 1,000 and 25,000 million bacilli per c.c. serum. The contents having been well mixed, the tubes were allowed to stand for two hours at room temperature (15° to 20° C.), and then placed for an hour at 54° C. to flocculate. They were then left overnight in the ice chamber, and next morning half an hour's centrifuging usually yielded a clear fluid. Only when a gross overdose had been used was it needful to centrifuge for many hours. The control tube was always kept with the others at all stages and served as the starting-point from which to calculate the reductions in titre: when all the estimations could not be done on one day, the control tube similarly served as a starting-point for the titres on the second day—a necessary precaution, as it was often found that the titre of the control had deteriorated somewhat.

The titres of the serum for some half-dozen selected strains were noted, and recorded as percentages of the titres in the control, whether these were high or low. The results could be plotted graphically in two ways: (a) by diagrams showing, to scale, the actual titres revealed before absorption and after each absorbing dose, or (b) by representing only the percentage changes—each titre in the control tube being reckoned as 100. We have used both forms, but the percentage diagrams are more compact, and on the whole, more instructive than the others, and though it is often

¹ This scheme of dosage does not necessarily apply to other species of bacteria. Much heavier doses are required with the enteric group of bacilli.

useful to compare the two in order to keep a proper mental perspective, we have used the percentage figures to illustrate this report.

We trust that this strictly quantitative method of working, and the diagrammatic mode of representing the results, constitutes an improvement on the methods of employing the absorption test which have hitherto prevailed. When once the plan on which the diagrams are constructed is understood, the facts they represent are more readily apprehended than from rows of figures. It has been possible to trace, by the means we have employed, the absorption from a single serum, by a single absorbing strain, of the agglutinins for any number of allied strains, while by introducing a number of different absorbing doses the process can be followed with any desired degree of closeness.

V. THE STUDY OF A SERIES OF STRAINS AS REGARDS AGGLUTINATION AND AGGLUTINOGENIC POWER.

Having described the methods by which we have worked, we now pass to the results obtained by the study of the 20 (later 21) strains which formed our starting-point. It will be convenient to refer to these strains by name.

1. *Standard strains of known type.*

Oxford Flexner.
Oxford 'Y'.
Lentz Flexner.
Lentz Y.
Lister Institute Hiss and Russell Y.

2. *Unselected and Unknown Strains.*

Cable (France):
Budden (Malta).

And 14 strains from carriers at Southampton, viz.:

Waller.
Bennett.
Stansfield.
Masom.
Whittington.
Coles.
Lee.
Hill.
Allam.
Mountain 2,558.
McCormick.
Hughes.
Huddart.
Mountain 2,464.

The fourteen unselected strains from Southampton were tested against the Oxford Flexner and 'Y' sera, and they were further cross-agglutinated with the sera from the fourteen patients them-

selves, which, as already mentioned, had been sent us with the cultures (see Appendix: 1).

Grouping was at once apparent. The Oxford Flexner serum agglutinated four out of the fourteen (Waller, Bennett, Stansfield, and Masom) to the limits of its titre, but affected none of the others to more than a quarter of its titre. The Oxford Y serum agglutinated two (Huddart and Mountain 2,464) to about its full titre, but affected the remainder very weakly. Eight strains were not agglutinated by either serum, but of these six were readily agglutinated by certain of the patients' sera as well or better than each serum agglutinated its homologous bacillus. These six strains were Whittington, Coles, Lee, Hill, Allam, and Mountain 2,558.

This rough orientation suggested the existence of a group corresponding neither with the Oxford Flexner nor the Oxford Y. We provisionally termed it the 'Z' group. Two strains (McCormick and Hughes) remained unaccounted for. The effects of three of the patients' sera are shown in Figure I, to illustrate the suggested grouping.

The next step was to prepare monovalent rabbit sera against as many strains as possible. Twelve such sera were made at this stage, more or less at random. The strains from which sera were prepared were Lentz Flexner, Lentz Y, Cable, Bennett, Masom, Whittington, Coles, Lee, Allam, McCormick, Hughes, and Mountain 2,464. We had also the Oxford Flexner and Y sera, and a little monovalent Hiss and Russell Y serum from the Lister Institute. Thus we had fifteen sera and twenty-one strains of bacilli.

Each of the fifteen sera was now in turn titrated against formalized emulsions of the twenty-one bacilli (see Appendix: 2) and the results, calculated as percentages of the titre of each serum for its homologous strain, were plotted out in diagrammatic form. A similar table, showing the results with other sera, is given in Appendix: 14. The existence of three groups was now manifest, and there was a suggestion of at least one additional group, which we termed 'X', while the irregular behaviour of the supposed 'Y's' made it possible that some further grouping existed here. By the use of appropriate colours the graphic charts took the form of 'spectra' for the individual sera and bacilli, in the manner already described. Study and comparison of the charts so obtained proved exceedingly instructive, for the character and properties of each serum and bacillus were immediately apparent. The charts could be sorted and grouped, and agglutination could be compared with agglutinogenic power. Examples of the charts are given at the end of this report (see Figures II-VIII, in which the spectra of seven sera and corresponding bacilli are shown together for comparison). The following conclusions were reached, and it will be convenient at this stage to refer to the different groups by the lettering ultimately adopted after our work, and that of others who had been engaged in the same task, had been reviewed by a committee of bacteriologists appointed by the War Office Committee on Dysentery. This committee, in order to avoid confusion with the lettering of Kruse's races and because the term 'Y bacillus' was already in existence, agreed to apply the last five letters of the alphabet to the five serological races established.

1. *V-group* (corresponding with the 'Flexner' strains of laboratories).

Six of the twenty-one bacilli fell into this group, viz. the Oxford Flexner and Lentz Flexner strains, and four of the Southampton races—Bennett, Masom, Waller, and Stansfield. Four sera had been made from members of this group—from the two known Flexners and from Bennett and Masom. These four sera all agglutinated the six bacilli named above to their full titre or thereabouts, but they agglutinated the remaining fifteen bacilli only weakly. The remaining eleven sera did not agglutinate the six bacilli to more than a quarter, or at most a half, of their full titre. The 'spectra' of the four sera and the six bacilli of this group resembled one another very strongly.

There was, however, a suggestion that these six *V*-strains fell into two minor groups. The Oxford and Lentz Flexner strains, and the Bennett strain were hardly agglutinated at all by sera of the 'Z' group, and their sera failed to agglutinate *Z*-bacilli to so much as one-fifth of their titre. The other three strains—Masom, Waller, and Stansfield—were agglutinated by *Z* sera to about half the titre of the latter, and Masom serum agglutinated *Z* bacilli to 40 per cent. of its titre. It will be seen later that this sub-grouping is justified by absorption experiments, and that there exists a sub-group of *V*-bacilli with a marked *Z* element in its composition (*VZ* sub-group).

2. *Z-group*.

Seven of the twenty-one bacilli fell into this group—Lee, Whittington, Coles, Hill, Allam, and Mountain 2,558 from Southampton, and Budden from Malta. From Lee, Whittington, Coles, and Allam sera had been prepared. These seven strains appeared to form a homogeneous group. Their own four sera agglutinated them all approximately to titre, but scarcely affected any of the other fourteen strains except the three members of the *VZ* sub-group which they agglutinated to about half their full titre. Similarly the remaining eleven sera had only a feeble effect upon the bacilli of the *Z* group, with the exception of the *X* sera which agglutinated *Z* bacilli to 60–70 per cent. of their own titre.

3. *X-group*.

Two strains, Hughes and McCormick, fell into this group, which was only tentatively recognized at this stage of the work. A serum had been prepared from each. These strains presented this striking peculiarity: they could be agglutinated only by their own and each other's sera; the remaining thirteen sera agglutinated them to no more than one-twentieth of the titre of each. Yet the sera prepared from these two strains freely agglutinated the *V* strains to 20 or 30 per cent., and the *Z* strains to 60 or 70 per cent. of their full titre. Thus in the case of the *X* bacilli, in place of the close resemblance between the 'spectra' of the bacilli and of their sera seen in the other groups, there was a striking divergence between agglutinability and agglutinogenic power (see Fig. V). Later work showed that the *X* strains deserve rank as a distinct race.

4. *W-group.*

Of the remaining six strains, three behaved alike, viz. the Oxford 'Y', Cable, and Huddart. We had two sera from these, viz. Oxford 'Y' and Cable, and these agglutinated the three bacilli to their full titre, but scarcely touched the other eighteen strains, with one exception, viz. Mountain 2,464 (not to be confused with the Z strain of the same name—Mountain 2,558). We shall later endeavour to show that Mountain 2,464 belongs to the *W* group, but to a peculiar sub-group of it in which a strong *X* element is present. The three bacilli here in question, Oxford 'Y', Cable, and Huddart, were not agglutinated to any noteworthy extent by any except their own sera.

5. *Y-group.*

The Lentz *Y* strain and the Lister Hiss and Russell *Y* behaved differently from all the preceding strains. They were agglutinated only to low titre by all the other sera, but their own sera agglutinated them well and each serum agglutinated the other bacillus to 40 or 50 per cent. of its titre.

At this stage of the investigation we did not know which of our alleged *Y* strains was the historical *Y*, though this claim was made for the Lister strain. The claim proved correct when we obtained from Washington a culture of the historical strain. The so-called Oxford *Y*, like the Mount Desert *Y* issued from the Rockefeller Institute, belongs to a different serological group for which we have had to use the term *W*.

*Summary of Evidence obtained from Agglutination and
Agglutinogenic Power.*

The actual figures obtained in the investigations summarized above will be found appended to this report (see Appendix: 2), and for the sake of the many readers to whom diagrams appeal more than rows of figures, we have added a sufficiency of coloured charts illustrating the 'spectra' of the different races of bacilli as regards agglutination and agglutinogenesis. These charts will, we think, show the reality of the serological differences between the various races outlined above, as revealed by the technique we used.

To us, at this stage of our inquiry, it appeared not unlikely that there were at least four antigenic components present in the Flexner group, viz. *V*, *Z*, *X*, and *W*, and that the predominance of each of these, singly, imparted its separate stamp to the races so lettered. We also recognized the probability that two other intermediate groups might exist, *VZ* and *WX*, in which two antigenic components might be present in sufficient amount to influence serological behaviour. As to the *Y* group, we were still largely in the dark.

It now remained to test these ideas by the application of the absorption method.

VI. THE ABSORPTION TEST APPLIED TO THE SAME SERIES OF STRAINS.

This test is based upon the fact, first observed by Castellani, that absorption of a serum with an adequate dose of the homologous

bacterium removes all the agglutinins, primary and secondary alike, whereas absorption with a different species removes only such secondary or group-agglutinins as are capable of union with the heterologous organism, leaving the so-called 'specific' agglutinin untouched. Group agglutinins are too often regarded as accidental by-products, and dismissed as of little account, or as an inevitable nuisance. It is, however, generally conceded that the formation of such group-agglutinins must be due to the presence, in the antigen employed, of subsidiary substances (proteins or lipoids) common to the organism used and to those coagglutinated.

When the phenomena of absorption are studied in relation to the serological races within the limits of a single species, secondary or group-agglutinins may assume considerable importance. For it is natural that the antigenic components of closely related races should themselves be more closely related than those of distinct bacterial species. It is a well-attested fact that when Dreyer's agglutination technique is employed, group-agglutination between distinct bacterial species is far less obtrusive than with the older methods of performing the test. We have used Dreyer's technique throughout, and in due course we shall describe such small degrees of coagglutination as we have found between Flexner's bacillus and other species. But within the limits of the Flexner species, group agglutination amongst the different serological races is a conspicuous phenomenon, and affords valuable evidence of antigenic relationship, and probably of the actual antigenic structure of individual strains. Nevertheless, although this group-agglutination is conspicuous amongst the Flexner races with Dreyer's technique, it is much less conspicuous than with other agglutination methods: in other words, Dreyer's method tends to emphasize the differences between the several races, whereas certain other methods tend to some extent to level them up.

The hypothesis of multiple antigenic components assumes the existence, side by side in the body of a given bacterium, of several distinct but related antigens, each of which in due proportion yields its distinct agglutinin when the bacterium is employed in immunization. That this is so is seen by comparing the 'spectra' of the different Flexner races with those of the sera they evoke: the only striking exception is in the case of the X-group. The problem whether, when a serum agglutinates its homologous bacillus, its titre depends upon the specific agglutinin only, or upon the resultant action of all the agglutinins present, is one not easy to settle. The partial reduction in specific titre for the homologous bacillus which often follows absorption by a heterologous strain would be explained on the latter view, but the fact that such an effect is limited and partial however large the absorbing dose, seems against the acceptance of such a simple quantitative explanation.

In practice, the unravelling of the antigenic structure of a bacterium by means of the absorption test is a very difficult task and one beset by many fallacies. The structure of the bacterium is complex, and the only substance by which we can test it, namely, the serum, is equally complex. It is as if we attempted to analyse a chemical mixture by means of reagents not themselves pure, but of equally unknown complexity with the mixture to be analysed. Neverthe-

less, we hope to show that the absorption test is of considerable utility in such an undertaking.

It offers, in the first place, a criterion of the individuality of a supposed antigenic component. If we have before us, let us say, four allied bacterial races, each of which we conjecture to be distinguished by the predominance of a special antigenic component, we can absorb the serum of one of the races with large doses of the other three, separately and even in combination. If the serum in question is left with its titre for its own race intact or only moderately reduced, good evidence is thereby afforded that this race has a special antigenic component of its own. This process can be repeated for each race in turn. It is on such lines that we have endeavoured to prove that the four races of Flexner's bacillus are in fact distinguished, as their agglutinating and agglutinogenic properties suggest, each by the presence of different predominating antigens.

In the second place, the absorption test offers convincing evidence as to the identity or non-identity of any two bacterial strains. One may have good grounds, from agglutination, for believing two organisms to be identical; but in our view invaluable support is lent to such an opinion by the results of absorption, and above all, as Kruse has well pointed out, by reciprocal absorption tests. On the principle established by Castellani, a serum should be exhausted of all its agglutinins by a homologous strain, but only of some of them by a heterologous one. It might be thought that a single absorption by an adequate dose would afford sufficient evidence, but cases occur in which this procedure is not enough. For, as Kruse pointed out, and as we ourselves have found, of two strains, A and B, A may be able completely to exhaust serum B, but B may be unable to do the same for serum A. It must be supposed that in such a case A contains not only all the antigens present in B but another in addition. Only when A can exhaust serum B, and B can equally exhaust serum A, can complete homology be considered established.

The method we have adopted of expressing our absorption results, in percentage form, as graphs, enables the homology or heterology of two strains to be appreciated at a glance. By testing the serum, before and after absorption, upon a number of different strains, the data for the presence or absence of the Castellani phenomenon are always present. By employing at least two absorbing doses, a small and a large, additional emphasis is furnished to the result. The small dose commonly produces a moderate or small fall in the agglutinins for some or all of the strains tested. The large dose may carry on the fall to virtual extinction or it may produce little or no further change. The graphic pictures by which we record our data come therefore under two general types (compare Fig. XI with Fig. X). In the case of absorption with a homologous bacillus the effect of the small absorbing dose is to cause the titres for the various strains tested to fall to varying extents, so that in the diagrams the lines fall fanwise from the 100 per cent. point at which they all start, *ex hypothesi*, in the unabsorbed serum. The large dose causes them to reconverge to a point near zero. In the case of absorption with a heterologous strain, the effect of the small dose is the same as before—a selective fanwise fall in the agglutinin content of the

serum for the various strains employed—but the large dose is seen to produce a further fall to zero only in those agglutinins with which it is in a position to combine: the titres of the serum for races alien to the absorbing strain show little or no further fall, and the lines representing them now pursue a horizontal course in the diagram. To use a very rough simile, the diagram in the case of a homologous absorption resembles the course of divergent rays of light brought to a focus by a biconvex lens, while in a heterologous absorption the effect simulates the further dispersal effected by a biconcave lens.

We will now state summarily the evidence which we have obtained from absorption experiments as to the existence in the Flexner group of the four distinct antigenic components which we were led to surmise from agglutination and agglutinnogenesis. The actual data will be found in full in the Appendix at the end of this report, and are summarized in a diagram (Fig. XVII).

It will be observed that, in presenting our evidence from absorption, we have relied mainly on arguments derived from the behaviour of the primary agglutinin—i.e. that for the strain homologous with the serum. Here we feel that we are on tolerably safe ground. The behaviour of the secondary agglutinins in absorption experiments, except in so far as they illustrate the Castellani phenomenon, offers a much more intricate problem, and we have thought it wise to refrain from drawing any conclusions. Nevertheless, the complete data are given in the Appendix: it may be that in the hands of others they may be made to yield useful information.

Evidence as regards the V Component (see Appendix: 3 and 4).

	<i>Serum.</i>	<i>Absorbing strain.</i>	<i>Dose in millions per c. c. serum.</i>	<i>Titre for homo- logous strain.</i>		<i>Percentage reduction of homo- logous titre.</i>
				<i>Unab- sorbed.</i>	<i>After absorp- tion.</i>	
<i>Homologous absorptions</i>						
(a)	Oxford Flexner	Oxford Flexner	12,800	3,312	697	79 %
(b)	Lentz Flexner	VZ Strain (Cahourg)	58,000	1,132	33	97 %
(c)	Oxford Flexner	VZ Strain (Masom)	716,000	3,112	140	96 %
<i>Heterologous absorptions</i>						
(d)	Oxford Flexner	Z Whittington	170,000	3,312	3,312	0
(e)	Lentz Flexner	X Hughes	12,500	1,763	1,763	0
(f)	Oxford Flexner	W Oxford Y	187,000	3,762	3,317	7 %
(g)	Lentz Flexner	W Cable	480,000	1,943	1,943	0
(h)	Lentz Flexner	{ X Hughes W Cable Z Whittington }	30,000 of each	2,500	1,656	33 %

TABLE II. ABSORPTIONS OF V SERA BY VARIOUS STRAINS.

These figures show that while the agglutinin for V strains is readily absorbed by V and VZ strains, it cannot be absorbed, or to a trivial extent only, by X, W, and Z strains separately, even in enormous doses. Heavy simultaneous absorption with X, W, and Z strains combined effects a reduction of only one-third in the titre of a V

serum for its homologous strain—a result probably due to secondary antigens in these strains capable of combining with the *V* agglutinin. The evidence clearly indicates that the *V* component and its corresponding agglutinin are distinct from *X*, *W*, and *Z*.

Evidence as regards the Z Component (see Appendix: 5 and 6).

	Serum.	Absorbing strain.	Dose in millions per c. c. serum.	Titre for homologous strain.		Percentage reduction of homologous titre.
				Unabsorbed.	After absorption.	
Homologous absorption						
(a)	Z Whittington	Z Whittington	18,640	1,556	69	95.6 %
Heterologous absorptions						
(b)	Z Whittington	<i>V</i> Oxford Flexner	17,200	1,250	1,250	0
(c)	Z Lee	<i>X</i> Hughes	221,000	1,656	1,250	25.2 %
(d)	Z Lee	<i>W</i> Cable	34,000	1,656	1,656	0
(e)	Z Lee	{ <i>V</i> Lentz Flexner <i>X</i> Hughes <i>W</i> Cable }	30,000 of each	2,500	1,456	41.8 %

TABLE III. ABSORPTIONS OF *Z* SERA BY VARIOUS STRAINS.

Here it is seen that the titre for *Z* is easily exhausted by a moderate saturation with the *Z* bacillus, but that *V* and *W* strains are without effect. A very heavy absorption with an *X* strain effects only a reduction of one-quarter in the titre for *Z*. Heavy absorption with *V*, *X*, and *W* strains combined removes rather more than two-fifths of the *Z* agglutinin. The *Z* component thus seems to deserve rank as a separate entity.

Evidence as regards the X Component (see Appendix: 7 and 8).

	Serum.	Absorbing strain.	Dose in millions per c. c. serum.	Titre for homologous strain.		Percentage reduction of homologous titre.
				Unabsorbed.	After absorption.	
Homologous absorptions						
(a)	<i>X</i> Hughes	<i>X</i> Hughes	12,800	2,500	250	90 %
(b)	<i>X</i> Hughes	<i>X</i> McCormick	20,000	1,656	31	98 %
(c)	<i>X</i> McCormick	<i>X</i> Hughes	20,000	1,250	250	80 %
Heterologous absorptions						
(d)	<i>X</i> Hughes	<i>V</i> Oxford Flexner	15,000	1,948	1,656	15 %
(e)	<i>X</i> Hughes	<i>Z</i> Whittington	15,000	1,948	1,656	15 %
(f)	<i>X</i> Hughes	{ <i>V</i> Lentz Flexner <i>W</i> Cable <i>Z</i> Whittington }	30,000 of each	1,557	697	55 %

TABLE IV. ABSORPTIONS OF *X* SERA BY VARIOUS STRAINS.

These figures show that *X* agglutinin is freely absorbed by *X* strains, but only slightly by *V* and *Z* strains. The effect of a *W* strain separately was not determined, as the *X* sera had scarcely an

appreciable titre for this race. The combined effect of large doses of *V*, *W*, and *Z* was a reduction of the *X* agglutinin by rather more than one-half—a reduction which was already in great part effected by a small dose of 1,000 million of each bacillus: there was little further fall with the large dose (see Fig. XIII). This reduction is greater than in the corresponding experiment upon *V*, *W*, and *Z* sera. We conjecture that this may be because the *X* strains are less pure in type than the other three: witness the marked agglutinogenic properties of the Hughes and McCormick strains as regards *V* and *Z* bacilli. Nevertheless, the facts support the contention that *X* represents a distinct antigenic component.

Evidence as regards the W Component (see Appendix: 9 and 10).

	Serum.	Absorbing strain.	Dose in millions per c. c. serum.	Titre for homologous strain.		Percentage reduction of homologous titre.
				Unabsorbed.	After absorption.	
Homologous absorption (a)	W Cable	W Cable	9,600	848	70	91.8 %
Heterologous absorptions	(b) W Cable	V Lentz Flexner	432,000	792	697	12 %
	(c) W Cable	VZ Cahourg	32,000	498	360	27 %
	(d) W Cable	X Hughes	50,000	697	697	0
	(e) W Oxford Y	Z Whittington	25,000	3,312	3,312	0
	(f) W Cable	{ V Lentz Flexner X Hughes	30,000 of each	697	657	6.8 %
		Z Lee				

TABLE V. ABSORPTIONS OF *W* SERA WITH VARIOUS STRAINS.

These figures show that the *W* agglutinin is readily absorbed by quite a moderate dose of the *W* bacillus, and is not absorbed at all by *X* and *Z* strains. A certain reduction is effected by *V* strains: the strain Cahourg, which reduced it by 27 per cent., was a *VZ* strain, not included in our original series, which was agglutinable by *W* sera to a distinct degree. Even in the last absorption (f) the reduction of agglutinin by large simultaneous doses of *V*, *X*, and *Z* is almost negligible. The existence of a distinct *W* antigenic component appears established.

Primary Races. The facts exhibited in the preceding four tables will be found summarized in the form of a diagram at the end of this report (Fig. XVII). The absorption test appears fully to support the surmise made on the basis of agglutination and agglutinogenesis. We seem justified in the belief that there exist at least four distinct antigenic components, and corresponding agglutinins, within the limits of the Flexner species, and this without including the true *Y* group, a consideration of which follows a little later. Where, as is usually the case, one of these components so far predominates over the others as to govern all the serological behaviour of a strain, we can group it accordingly, speaking of *V*, *W*, *X*, and *Z* races. The *V*, *W*, and *Z* races are fairly well defined both as regards agglu-

tination and agglutination. The *X* race is the most sharply defined of all as regards agglutination, but the least sharply as regards agglutination: its specific agglutinin is also absorbed by alien races to a somewhat greater degree than that of the other three races.

Composite Races. The existence of some degree of coagglutination amongst these races suggests that no one of them is built up of a single antigenic component: to some extent the other components are probably present in all. And the classification above suggested does not quite embrace all the facts we have elicited. We have found evidence of the existence of composite races in which a second antigenic component enters so largely into the structure of the bacterium as to influence its serological behaviour to a marked degree, without, however, sufficing to remove it from the main race to which it belongs. We have been able to work out the characters of two such sub-races, and it may be that others exist. The two which we have studied appear well defined and constant in their behaviour and not mere haphazard forms; both have been fairly common during the war, indeed one has been amongst the commonest types met with in France. The first is a variety of the *V*, and the second of the *W* race; that is to say, one has *V* and the other *W* as its main antigenic component. The *V* contains a *Z* component, and the *W* an *X* component as an additional governing element. Allusion has already been made to the probable existence of *VZ* and *WX* sub-races in discussing the evidence from agglutination and agglutination; it will be convenient now to summarize the evidence in each case.

Evidence as regards the VZ Sub-group (see Appendix: 11).

Three of our series of twenty-one strains fall into this group (Masom, Waller, and Stansfield), but we have met with many others in our extended series of strains. With a *V* serum they all agglutinate to the same full titre as the pure *V* strains, but differ from them in agglutinating to a much higher degree with *Z* sera. The following table exhibits the effect of four *Z* sera upon three pure *V* and three *VZ* strains, the figures being *percentages* of the titre of each *Z* serum for its homologous bacillus:

<i>VZ strains.</i>					<i>V strains.</i>		
	<i>Masom.</i>	<i>Waller.</i>	<i>Stansfield.</i>		<i>Oxford Flexner.</i>	<i>Lentz Flexner.</i>	<i>Bennett.</i>
<i>Z</i> serum Coles . . .	23	53	53		4	5	5
„ Allam . . .	20	44	46		4	5	7
„ Lee . . .	20	28	39		4	4	5
„ Whittington . . .	26	45	56		5	5	6

TABLE VI. RELATIVE AGGLUTINABILITY OF *VZ* AND *V* STRAINS BY *Z* SERA.

Similarly our *VZ* serum (Masom) agglutinated seven *Z* bacilli to an average of 35 per cent. of its full titre for its own strain, whereas three *V* sera agglutinated them only to an average of 16, 10, and 8 per cent. respectively. Thus the evidence from agglutination

and agglutininogenesis suggests a differentiation of the *V* race into two sub-groups, which can in practice be distinguished by employing a *Z* in addition to a *V* serum.

The evidence is, however, stronger than this. The absorption test has afforded us the following data. Large doses of *VZ* bacilli can exhaust a *V* serum of practically all its *V* agglutinin: they cannot remove from a *Z* serum more than a part of its *Z* agglutinin (29 per cent. only, in an experiment in which a *Z* serum was treated with 30,000 million *VZ* bacilli per c.c.). Thus they must be regarded as much more closely related to the *V* than to the *Z* race. A similar conclusion was reached from a converse experiment in which a *VZ* serum (Masom) was treated separately with 90,000 million doses per c.c. of *V* and *Z* bacilli, and also with a mixture of the two (45,000 million of each) (see Appendix: 11 b, and Fig. XIV). The *V* bacillus alone reduced the *VZ* agglutinin by 78 per cent., the *Z* bacillus alone by 53 per cent., and the combination of the two by 80 per cent. The graphs of these absorptions are given at the end of this report, and it will be seen that while the absorption with *Z* is of the heterologous type, that with *V* approaches the homologous type, while that with *V* and *Z* combined is almost exactly like a homologous absorption.

A still more striking experiment was the following. A *Z* serum (Whittington) was absorbed with a pure *V* bacillus (Oxford Flexner) in doses up to 17,000 million bacilli per c.c. serum. Before absorption this serum had a titre of 25 for two *V* strains (Oxford and Lentz Flexner): for two *VZ* strains (Stansfield and Masom) its titres were 697 and 500: for four *Z* strains (Whittington, Lee, Allam, and Mountain 2,558) its titre varied from 849 to 1,768. After absorption the small titre for the pure *V* strains was wholly extinguished, but the titres for the two *VZ* strains, and for the four *Z*'s, were wholly unaffected. This experiment was repeated, with a similar result (see Appendix: 6 a and b).

We may thus regard the *VZ* type as a definite sub-group of the *V* race, behaving fundamentally as a *V*, but differing in the fact that it contains a sufficiently large *Z* component to modify its serological behaviour.

Evidence as regards the WX Group (see Appendix: 12).

Only a single strain amongst the twenty-one studied falls into this sub-group, but in our extended series we have met with a number of others, about a dozen in all, and they appear to form a definite group with constant characters. The strain fully studied, Mountain 2,464, was agglutinated by one of our *W* sera (Oxford 'Y') nearly to its full titre, but by the other *W* serum (Cable) not quite to half of its full titre. But, unlike other *W* strains, it was also agglutinated by an *X* serum (Hughes), nearly to the full titre of this serum. Now it is a peculiarity of all the *X* sera which we have used (only three in all) that they will not agglutinate *W* strains to more than a minute fraction of their titre, and this exception to the rule aroused our attention. The strain in question, Mountain 2,464, was not agglutinated by sera other than *W* and *X* to more than a small degree,

with the exception of one *Z* serum (Lee) which agglutinated it to 40 per cent. of its titre.

The serum prepared with the Mountain 2,464 strain was very specific, no other strain being agglutinated by it to more than about one-fifth of its titre for itself.

The antigenic structure of the strain was elucidated by absorption tests. It was unable to absorb *Z* agglutinin to more than a very small extent. In a dose of 20,000 million per c.c. it reduced the *W* agglutinin in a *W* serum (Cable) by 79 per cent. In a dose of 30,000 million per c.c. it reduced the *X* agglutinin in an *X* serum (McCormick) by 36 per cent. Conversely, the agglutinin for Mountain 2,464 was reduced by 60 per cent. when its serum was absorbed with a *W* strain (Cable) in a dose of 20,000 million per c.c., and by 36 per cent. when absorbed with a 30,000 million dose of an *X* strain (McCormick). There is thus evidence of considerable antigenic relationship with the *X* race, and still more closely with the *W* race. The final proof was afforded by simultaneous absorption of the Mountain 2,464 serum with equal doses (25,000 million per c.c. of each) of *W* and *X* strains (Cable and Hughes). This reduced the titre of the serum for its own strain by 94 per cent. The graph obtained was practically identical with that yielded when the serum was absorbed with its own strain (Fig. XV).

There seems therefore sufficient ground for regarding this sub-group of the *W* race as a distinct one, in virtue of its large *X* element. But we were able to show in later experiments that it is truly a *W* and not an *X*. An emulsion of Mountain 2,464 was put up against mixtures of *W* and *X* sera in varying proportions, and the degree to which it was agglutinated ran parallel with the amount of *W* in the mixed sera and not with the amount of *X* (see Appendix : 13).

It is noteworthy that the *WX* serum was highly specific for its own sub-group, and did not agglutinate the *W*'s to more than about one-fifth of its full titre, or the *X* group to more than one-twentieth of its full titre. In this respect the *WX* type differs from the *VZ* type, for *VZ* sera freely agglutinate *V* and *Z* strains. In other respects these two sub-groups present analogous characters.

VII. THE STUDY OF THE Y GROUP.

Although we recognized later that two genuine *Y* strains had been present amongst the twenty-one strains originally selected for study, namely, the Lentz *Y* and the Lister Hiss and Russell *Y*, we were for a long while in considerable doubt about their nature and relation to our other strains. They have proved a more difficult group to elucidate than any of the others, and we still feel a good deal of uncertainty about their precise antigenic structure.

We were at length able to obtain an authentic culture of the original Hiss and Russell *Y* strain, through the kindness of the American Museum of Natural History: it was sent from Washington at their request by General Russell himself. Another *Y* strain was procured shortly afterwards from Captain Murray, R.A.M.C.: it had been given him by Lieut.-Colonel Ledingham in Mesopotamia, but he did not know its origin. Sera were prepared both from the

Washington Y and the Ledingham Y. We now had four Y strains with their corresponding sera. They proved closely allied but not absolutely identical with each other.

Agglutination (see Appendix: 2 and 14). The four strains were all well agglutinated by the Ledingham Y serum: the Lentz Y strain to 69 per cent., the Lister Y to 88 per cent., and the Washington Y to 94 per cent. of the full titre of the serum for its own strain. The Lister Y serum agglutinated the Ledingham Y strain to full titre, but the Lentz Y and Washington Y to half titre only. The Washington Y serum agglutinated the Ledingham Y practically to its full titre, but the Lentz and Lister Y's only to half titre. The Lentz Y serum agglutinated the Ledingham Y to full titre, the Washington Y to 82 per cent., and the Lister Y to 74 per cent. of its full titre.

The four Y's were thus well agglutinated by other Y sera, but they were very little affected by V and Z sera, and as a rule only weakly by X and W sera. One X serum, out of three tested, brought down the Lister and Lentz Y's to 56 and 22 per cent. of its full titre respectively, and one W serum out of four tested brought down the Lentz and Ledingham strains to 45 per cent. of its titre, the Lister strain to 87 per cent., and the Washington Y to full titre. It must be remembered that our emulsions were not standardized for agglutinability; had they been so these figures might have required considerable correction. The Washington Y was an extremely agglutinable strain. The general evidence from agglutination was, on the whole, such as to separate the Y's, as a group, from the V, W, X, and Z groups.

Agglutinogenesis. The evidence here was in the same direction. All the four Y sera agglutinated Y strains better than they agglutinated the preceding four races, as may be seen from the figures given at the end of this report. But they were not sharply specific; they usually agglutinated V strains to 40-50 per cent. of their full titre, Z strains to some 20 per cent., and X strains to some 10 per cent. of their titre; W strains were agglutinated to about 40 per cent. of full titre by the Washington Y serum, but not so well by the other three. There was thus, in these sera, a tendency to an all-embracing character as regards other races. This coincides with practical experience during the war; until nearly the end, when specific sera were supplied from Millbank, workers abroad had been obliged to rely on certain stock sera put out by various institutes, and of these by far the most useful—in the sense of giving the fewest negative results—was the Y serum sent out by the Lister Institute. This was the experience related to us by Lieut.-Colonel C. J. Martin, yet as a matter of fact, true Y strains were practically absent in France, though commoner on the Eastern fronts and in Italy.

Thus the general impression derived from the facts of agglutination and agglutinogenesis in our four Y strains, was that, while they formed a distinct serological race, they were a less homogeneous one than V, W, X, and Z, differing somewhat amongst themselves, and apparently presenting a greater mixture of antigens.

Absorption Experiments in the Y Group.

Our previous experience with the absorption test led us to hope that, by using the same methods, we should be able to determine the antigenic structure of the Y group as we believed we had done in the case of the other four groups. We hoped to be able to define a fifth antigenic component which might account for the racial peculiarities of the Y's, though we expected to find it accompanied by a considerable admixture of other components. This hope has not, however, been fulfilled: we have been unable to define a fifth component with any certainty, though we are by no means assured that it does not exist.

The lack of complete identity between some of the individual strains was shown by the reciprocal absorptions which we carried out in certain cases (see Appendix: 15).

The Lentz Y serum was absorbed with a big dose of the Lister Y bacillus, and was largely exhausted of its Y agglutinin. A dose of 74,000 million bacilli per c.c. reduced the titre for Lentz Y itself by 86 per cent., the titre for the absorbing strain (Lister Y) by 98 per cent., and that for the Washington Y by 97 per cent. The graph (Fig. XVI) is on the whole that of a homologous absorption.

But the reciprocal experiment, in which the Lister Y serum was absorbed with an even larger dose (77,000 million per c.c.) of the Lentz Y bacillus, gave a different result. The titre of the Lister Y serum for its own bacillus was reduced only by 45 per cent., that for the Washington Y bacillus by 75 per cent., while that for the absorbing strain fell to zero. The graph (Fig. XVI) is that of a partially heterologous absorption. Here, then, is an example of two allied strains in which the serum of the first can be largely exhausted by absorption with the second, but in which the serum of the second can only be partially exhausted by absorption with the first. The most probable explanation is that the Lentz Y strain is of simpler antigenic constitution than the Lister Y strain: the latter may contain all the antigens of the Lentz Y, and something in addition.

Somewhat different results were obtained in reciprocal absorptions between the Lentz Y and Washington Y strains, for here each bacillus proved able to absorb from the serum of the other 74 per cent. of the specific homologous agglutinin.

The search for a possible fifth antigenic component in the Y group was commenced by absorption experiments upon the Lentz Y strain (see Appendix: 16). A Lentz Y serum was absorbed with doses of V and W strains separately and combined. A dose of 32,000 million Lentz Flexner bacilli reduced the titre of the Lentz Y serum for itself by 58 per cent.: a similar dose of the Cable bacillus (W) reduced it only by 12 per cent. Simultaneous doses of the two (32,000 million of each) produced a slightly less effect than the Lentz Flexner alone, viz. a reduction of 52 per cent. Finally the Lentz Y serum was attacked with simultaneous doses of V, W, X, and Z (Oxford Flexner, Cable, Hughes, and Whittington)—32,000 million of each per c.c. of serum: this absorption brought down the Lentz Y titre for itself by 78 per cent., its titre for the Lister Y by 82 per

cent., and its titre for the Washington Y by 88 per cent. It will be remembered that when sera of the V, W, X, and Z races were similarly attacked, each by the other three combined, a large amount of the specific agglutinin—ranging from 94 per cent. in the case of the W serum to 55 per cent. in the case of the X serum—remained unabsorbed. Here we have only 22 per cent. of the agglutinin remaining.

The Lister Y serum was investigated in a similar manner (see Appendix: 16). A V strain (Lentz Flexner), in a dose of 25,000 million per c.c., absorbed 60 per cent. of the specific Lister Y agglutinin, at the same time reducing the titre for the Lentz Y strain by 81 per cent. A Z strain (Whittington), in a similar dose, absorbed 23 per cent. of the specific Lister Y agglutinin, and 27 per cent. of the agglutinin for Lentz Y. A W strain (Cable), in the same dose, absorbed 37 per cent. of the specific Lister Y agglutinin, and 30 per cent. of the agglutinin for Lentz Y. Finally the Lister Y serum was absorbed with simultaneous doses of V, W, X, and Z, rather smaller than had been used with the Lentz Y serum, viz. (per c.c. of serum) 10,000 million each of Oxford Flexner, Whittington, and Cable, and 7,500 million of the X strain—Hughes. The effect was to reduce the titre for Lister Y by 50 per cent. only, the titre for Lentz Y by 74 per cent., and that for the Washington Y by 77 per cent.

The Lister Y bacillus was also tested as an absorbing strain upon certain sera of other types. In a dose of 25,000 million per c.c. it failed to absorb any V agglutinin from the Lentz Flexner serum. In a similar dose it absorbed 50 per cent. of the Z agglutinin from the Whittington serum, and again in a similar dose it absorbed 47 per cent. of the W agglutinin from the Cable serum.

Conclusion. It must be confessed that these absorption experiments on the Y race do not lead to the same convincing conclusions as the similar experiments with the V, W, X, and Z races. They do, however, in a measure confirm the suggestion derived from agglutino-genesis and agglutination, namely that the Y race presents a mixture of antigenic components in which there is no striking predominance of any one component over the rest. The greatest reduction which can be effected in the specific titre of a Y serum is by absorption with a V strain, and this corresponds with the fact that Y sera agglutinate V strains better than any other alien races. It seems fair to conclude that the V antigenic component takes a larger share in the structure of the Y bacilli than the Z and W components while the X component plays but a small part.

Since, however, we did not succeed in absorbing the whole of the Y agglutinin with bacilli of the other four races combined, and indeed, in the case of the Lister Y serum, only 50 per cent. of it, it may be argued that the Y strains do present a fifth component. If so, it must form less than a quarter of the total in the case of the Lentz Y strain, and possibly less than a half in the case of the Lister Y, for the multiple absorption of the latter serum was not carried out with large doses.

In a recent paper, Murray has claimed a relationship between the Lister Y strain, with which he worked, and the lactose-ferment-

ing strains of the dysentery group, and he goes so far as to group the Lister Y with the latter. With such a conclusion we are in disagreement. In our hands the Lister Y strain failed to ferment lactose, and in the following experiments we were unable to confirm the somewhat close serological relationship which Murray asserts between the Lister Y and the lactose fermenters. By his kindness we were furnished with cultures of the strains from which he had prepared his sera, and from several we made sera of our own. Amongst these was the strain which he calls 'Ledingham Flexner', since it was given him in Mesopotamia by Lieutenant-Colonel Ledingham. It is a strain which we should exclude from the true Flexner group, not only because it ferments lactose and gives heavy acid agglutination, but because we can trace only a slight serological relationship with the true Flexners. Nevertheless, it seemed possible, in view of Murray's statements, that the missing fifth component in the Y group might be one found in the lactose fermenters. We made a serum from this 'Ledingham Flexner' strain, and it proved one of high titre—1 in 12,800. Like the other lactose fermenters we have examined, this strain differs from the true Flexner group in the very slow velocity of its agglutination: at the end of $4\frac{1}{2}$ hours little or no effect is produced by the serum, but after 20 to 24 hours at 55° C., the full titre is apparent. In the following experiments this time was therefore allowed:

The 'Ledingham Flexner' serum was put up against itself and 13 Flexner strains. After $4\frac{1}{4}$ hours at 55° C. the maximum agglutination on its own strain was only at a dilution of 1 in 28, but several of the Flexners showed nearly the same. Next morning, after 22 hours at 55° C., the titre of the serum was found to be 1 in 12,800 for its own strain, but no Flexner strain was agglutinated beyond 1 in 56. The maximum effect of this serum upon true Flexners was only 0.4 per cent. of its titre for itself.

An emulsion of the 'Ledingham Flexner' strain was now put up against 13 true Flexner sera, each serum being at the same time put up against its own strain as a control. The tubes were left 24 hours at 55° C. The Lentz Y serum agglutinated it to 1 in 1,100, or 52 per cent. of its full titre; three other Y sera agglutinated it to 12, 8, and 3 per cent. of their full titres. One Z serum (Whittington) agglutinated it to 19 per cent. of its full titre, and another Z to 10 per cent. The effect of V, W, and X sera was very slight—5 per cent. to 0.4 per cent. of their full titres.

The 'Ledingham Flexner' serum was next separately absorbed with large doses (50,000 million per c.c. in each case) of a Z strain (Whittington) and four Y strains separately (Lister Y, Lentz Y, Ledingham Y, and Washington Y). No absorption of the specific agglutinin took place; in all five experiments the titre of the serum for its own bacillus was the same in the absorbed tubes as in the unabsorbed controls.

It was concluded from these experiments that there was very little antigenic relationship between the lactose-fermenting 'Ledingham Flexner' strain and the true Flexner group, and that the hypothetical fifth component of the Y strains could not be looked for here.

We therefore consider that we have failed fully to solve the problem of the antigenic structure of the Y races. All that we have been able to do has been to obtain evidence of the presence in this race of most or all of the antigenic components which we have termed V, W, X, and Z, and of these V seems the best represented and X the least. Whether there is something more, we do not feel that we can be sure.

If the Y's represented merely a mixture of the four antigenic components named, in varying, but not very dissimilar proportions, no one predominating, we should have some sort of explanation of their dissimilarity, from a serological point of view, from the more specialized V, W, X, and Z races, in which one component has developed out of all proportion to the others. We might even hazard the speculation that, from a phylogenetic point of view, the Y races represented the most primitive members of the Flexner species, and the nearest allies to the less pathogenic members of the dysentery group as a whole.

One further experiment may be mentioned: it was undertaken in the hope of shedding further light on this question, but proved inconclusive in its results. The Lentz Y strain was selected as apparently the simplest in its structure, containing no appreciable X element. An attempt was made to construct an artificial Lentz Y serum by mixing V, W, and Z sera in equal parts. The results were as follows:

V. Lentz Flexner Serum alone.

Titre for Lentz Flexner	.	.	.	1,250
„ „ Lentz Y	.	.	.	90

Z. Whittington Serum alone.

Titre for Whittington	.	.	.	500
„ „ Lentz Y	.	.	.	50

W. Cable Serum alone.

Titre for Cable	.	.	.	300
„ „ Lentz Y	.	.	.	33

V, Z, W. Equal Parts of the three Sera.

Titre for Lentz Flexner	.	.	.	317
„ „ Whittington	.	.	.	331
„ „ Cable	.	.	.	125
„ „ Lentz Y	.	.	.	125

It will be seen that the mixture of the three sera agglutinated the Lentz Y strain in higher dilution than did any one of the sera separately. The mixture should have taken the Lentz Y to a dilution of

$\frac{90+50+33}{3} = 58$: actually it took it up to 1 in 125: there

was thus some evidence of summation of effect, though not of a very striking nature. We recognize that such experiments are subject to fallacy, and we do not feel able to lay much stress on this one.

VIII. THE PRESENCE IN FLEXNER SERA OF AGGLUTININS FOR BACILLI OF OTHER SPECIES.

It is well recognized by those who have worked with Dreyer's method of agglutination that coagglutination of other species is manifested to a far lesser degree with this technique than with other methods. Our observations on group agglutination will not therefore overstate any possible antigenic relationships between Flexner's bacillus and allied species.

Fourteen of the monovalent sera with which we have worked have been tested as regards their titres for a classical strain of *B. coli communis*, for *B. typhosus*, *B. paratyphosus A* and *B. Shiga's* bacillus, a supposed dysentery bacillus of the 'Schmitz' type, a lactose-fermenting bacillus of the dysentery group, and the dulcitate-fermenting *B. alkalescens*. The agglutinations were carried out in a water bath, with formalinized emulsions and a time limit of four hours; inasmuch as the two species last named have a low velocity of reaction, the titres obtained with them presumably understate the case. The sera employed had titres for their homologous strains varying from 1,000 to 3,000. The results are shown in the following table, which gives the actual titres observed or calculated, and not the percentages of full titre as in many of our tables:

Type.	Serum.	<i>B. coli communis</i> .	<i>B. typhosus</i> .	<i>B. paratyphosus A</i> .	<i>B. paratyphosus B</i> .	<i>Shiga</i> .	<i>Schmitz' type</i> .	Lactose fermenter.	<i>B. alkalescens</i> .
V	Oxford Flexner	0	0	0	0	0	0	17	80
"	Lentz Flexner	0	0	0	0	0	67	65	0
"	Bennett	0	0	0	0	0	25	176	0
VZ	Masom	0	0	0	0	0	50	74	36+
Z	Whittington	0	0	0	17	0	70	74	25
"	Coles	0	0	0	0	0	0	0	0
"	Lee	0	0	84	0	0	50	70	0
"	Allam	0	0	0	0	0	17	0	50
X	Hughes	0	0	0	0	0	31	69	0
"	McCormick	0	0	0	0	0	0	39	0
WX	Mountain 2,464	27	0	0	0	0	74	35	0
W	Oxford Y	35	0	0	0	0	0	31	31
"	Cable	0	0	0	165	0	50	67	69
Y	Lentz Y	0	0	0	33	68	0	25	0

TABLE VII. SHOWING AGGLUTINATING POWERS OF FOURTEEN FLEXNER SERA UPON OTHER SPECIES.

The first five columns of this table show that, tested by Dreyer's method, Flexner sera of fair potency derived from the rabbit rarely contain coagglutinins for *B. coli*, for members of the enteric group, or for Shiga's bacillus. Such coagglutinins are present here and there in apparently haphazard fashion, but only to a small fraction of the titre of the serum for its homologous strain. The absence of agglutinin for Shiga's bacillus in all but one serum is noteworthy.

The last three columns of the table show that some degree of agglutinating power is far more commonly present against three species more closely related to the Flexner group, though, as we

believe, distinct from it. Here too, however, the titres are low, only in one instance surpassing one-tenth of the full titre of the serum, though they would probably have been higher had a longer period been allowed for the reaction. It is true that many Flexner sera exhibit titres as low, or lower than these for certain other Flexner races; but it will be remarked that these agglutinins for other species are uniformly low in all the fourteen sera tested, which is never the case for a true Flexner strain; one or more of the sera of such a wide range as these always takes the latter to titre or nearly so. Nevertheless, the facts suggest some degree of antigenic relationship, even if a small one, between the Flexners and these allied species. In a few experiments previously carried out by one of us it had appeared that even heavy absorption with *B. alkalescens* failed to reduce the titre of a Flexner serum, except for *B. alkalescens* itself. In a recent experiment it was found that a Z serum exhausted with the homologous bacillus had suffered no reduction in its small, but easily measurable, titres for *B. paratyphosus* B, *B. alkalescens*, and a lactose-fermenting strain.

We also carried out an elaborate experiment to ascertain whether any non-specific absorption occurred in a Flexner serum saturated with a totally heterologous bacillus. A W serum (Cable) was absorbed with a classical strain of *B. coli communis* from the urine, in culture for two or three years. The doses employed were 500, 10,000 and 62,400 million per c.c. serum. This serum did not agglutinate the absorbing strain, and more than ten hours in a high speed centrifuge were required to clear the fluid. The titres were taken before and after these absorptions against V, W, and Z strains of Flexner, against *Paratyphoid* B, and against a 'Schmitz' strain, *B. alkalescens* and a lactose fermenter, but although some of the original titres were quite small, all of them were entirely unchanged even by the heaviest saturation with *B. coli*. This experiment appears to exclude any mechanical adsorption of agglutinin by a heterologous bacillus which cannot effect a true combination with it.

IX. SUMMARY OF THE CONCLUSIONS REACHED IN THE DETAILED STUDY DESCRIBED ABOVE.

As already stated, we commenced our work with a series of strains, numbering 20 or 21, and mostly unselected, deeming it best to study a few strains fully, and then to apply the conclusions reached to the larger series we had collected. Before passing on to this larger series, it will be convenient to give a short summary of our facts and conclusions.

1. We have defined the Flexner species, perhaps somewhat arbitrarily, not only by its fermentative reactions and serological relationships, but also by the results of the acid agglutination test. It is not denied that this test may exclude certain bacilli which may be concerned in the causation of dysentery, e.g. certain lactose-fermenting types, but we believe that such organisms belong to distinct species from Flexner's bacillus, and we are concerned, in this paper, with the latter alone, which appears more important from the point of view of the epidemic disease.

2. We have brought forward evidence, based on agglutination by monovalent rabbit sera, on agglutinnogenesis and on absorption, which indicates the presence, in the members of the Flexner species, of at least four distinct antigenic components, all of which are commonly represented in any given strain, but to a very different degree. In certain races which we designate *V*, *W*, and *Z*, there is so great a preponderance of a single antigenic component, different in each case, that these races behave, serologically, almost like distinct species, and each requires a serum belonging to its own race for its adequate agglutination. We have found also a fourth race, *X*, which is peculiar in that it refuses to agglutinate with any but sera of its own race, save to a trivial degree, but yet is able to give rise to a serum which will agglutinate not only *X* races, but also *Z* races almost as well, and to some extent *V* races. We have brought forward evidence that the agglutinins corresponding to each of these four antigenic components cannot be more than partially absorbed by the others, and we believe them to be distinct entities.

3. We have also endeavoured to show that at least two sub-races exist, which we term *VZ* and *WX*, essentially members of the *V* and *W* races respectively, but containing so large a proportion of a second antigenic constituent as to modify their serological behaviour.

4. We find the true *Y* race to show different serological characters from any of the preceding, so that it also requires a serum of its own race for adequate agglutination. We have so far been unable to feel sure that it contains a fifth antigenic component, but we believe that it presents a mixture of the *V*, *W*, and *Z* components, and to some extent the *X* component also, more evenly balanced than in the case of the other races, so that a *Y* serum has a wider range of agglutination than other Flexner sera. We hazard the conjecture that the *Y* race is of a more primitive antigenic structure than the rest.

5. Inasmuch as we have found every race examined to present more than one antigenic component, there is some degree of overlapping amongst the different races. We have, however, found the races defined, even by agglutination alone, more sharply than some other workers have found them. We attribute this chiefly to the use of Dreyer's agglutination technique, which minimizes coagglutination.

X. COMPARISON OF THE RACES ABOVE DEFINED WITH THOSE ESTABLISHED BY OTHER WORKERS.

It is unfortunate that, owing to the war, we have been unable to obtain any examples of the serological races of this group established by Kruse. We have, however, been in touch with the work of two other observers who were engaged on a similar task at the same time that we were working. The late Dr. H. S. Gettings was working at St. Mary's Hospital, under the Medical Research Committee, while Captain E. G. D. Murray, R.A.M.C., was working at the Royal Army Medical College. Captain Murray has published his results in the *R.A.M.C. Journal*, and the work of Dr. Gettings,

collected after his death by Captain S. R. Douglas (I.M.S. retired) has been published by the Medical Research Committee. Owing to the fact that a mutual interchange of cultures took place between both these observers and ourselves we are in a position to compare their results with our own, although the work and conclusions were in each case entirely independent.

Dr. Gettings's material consisted of nearly a hundred strains derived largely from outbreaks of asylum dysentery at Wakefield, but also in part from the War Hospital at Epsom. He prepared four sera, but found so much overlapping of races that he was unable to formulate any sharp classification from agglutination alone. When, however, he turned to the absorption test, he found that his strains fell into four perfectly sharp groups. We have been furnished by Captain Douglas with type strains and sera of these four groups, and have tested the strains with our own sera, and the sera on our own strains. We find that one of his races (Hermann) corresponds exactly with our *V*, and another (Clowes) with our *Z* race. A third (Howden) is identical with our *W* race in essential respects, but its serum differs from our *W* sera in agglutinating *Y* races also to a considerable degree. Gettings's fourth race (Pearce) is one which we have excluded from the true Flexner species on the grounds that it yields strong acid agglutination and is serologically unconformable with other members of the Flexner group, though its sugar reactions are identical. This race, in Gettings's opinion, was responsible for a definite outbreak of dysentery at Wakefield. Of the three races which we include in our Flexner group, two are absolutely and the third practically identical with races we have independently defined.

Captain Murray's work covered a larger field than ours, inasmuch as he dealt with dysentery bacilli of all kinds. We are here concerned only with his classification of the Flexner group, of which he examined thirty-three strains. Using the same general methods as ourselves, but a different technique, he found his strains to fall into four groups, which he does not consider sufficiently sharply defined to be regarded as separate races. We possess cultures of his four races and have made sera from three of them. One of his races is absolutely identical with our *V*, another with *W*, a third with *Y*, while the fourth corresponds with our *X* and *Z*, between which Murray has drawn no distinction. In comparing Murray's classification with our own, it is apparent that it is almost identical. The chief points of divergence are easily explained. The amount of overlapping which he found amongst his races and which induced him to refuse them full recognition, depends, in our judgement, upon difference of agglutination technique. He employed living agar suspensions, with a twenty-four-hour incubation at 55° C., and complete sedimentation as his end point: he did not in all cases pursue the agglutination to its final end point. These details are all liable to level up differences between allied races, whereas Dreyer's method produces the opposite effect. The association of our *X* and *Z* races in a single group by Murray, is due to the fact that he did not prepare a *Z* serum. His *X* serum agglutinated his *Z* strains, as was the case with ours, and he was satisfied with this; had he made a *Z* serum we believe that he would have found that it would not agglutinate his *X* strains. We were

so fortunate as to make both. Another point in which we find ourselves in disagreement with Murray, lies in his exclusion of the Lister Y from the Flexner group: to this we have previously referred.

It is nevertheless a satisfaction to us to find such general agreement between our results and those independently reached by Dr. Gettings and Captain Murray. When separate observers, working on largely different material, reach conclusions so similar as these, the probability that the conclusions are correct is largely increased.

The Position of certain well-known Strains in our Classification of Flexner Races.

In the course of our work certain well-known strains, or strains kept as types in well-known laboratories, have, by the kindness of friends, come into our hands. It may be of service, for purposes of correlation, to indicate the groups into which these fall on the racial classification we have put forward. The following statements are based for the most part on agglutination results with our type sera:

Our V race corresponds with most of the strains kept as typical 'Flexners' in European laboratories. Thus the Lister Institute Flexner, the Flexner of the Oxford Laboratory, and that from Lentz's laboratory in Berlin are all typical V's. The 'd'Herelle' strain which we received from the Institut Pasteur appears to fall into our VZ group and so also does Dudgeon's 'Gallipoli' strain.

No well-known strain which has reached us falls either into our X or Z groups. The so-called Y of the Oxford Laboratory belongs to our W group, as does the Mount Desert Y sent out by the Rockefeller Institute. A strain termed 'Strong', stated to have been isolated by Strong in Manila many years ago, and sent us by the American Museum of Natural History, is also a W. From the same source we received the 'Flexner Harris' strain; it is certainly not of the V race, but is either a W or a true Y, being agglutinated by both W and Y sera almost equally: without testing its agglutinogenic and absorptive powers we are uncertain in which group to place it. Dudgeon's 'Logan' strain is a typical W.

Taking as our type Y the strain received from General Russell in Washington, we find the Lister Institute Y and the Y from Lentz in Berlin to correspond with this. We have felt bound to retain the term 'Y' for the race, because Hiss and Russell were the first to employ it, and it has therefore historical priority, although the Mount Desert strain was, we are informed, isolated (though not called a Y) a year before Hiss and Russell isolated and named their strain.

At the time when the Flexner and Y races were first isolated in the United States and Manila, considerable confusion, or, rather, unavoidable ignorance must have existed as regards the different races. One race, which happened to ferment saccharose, and was serologically different from the others, came to be known as the 'Strong' type, and if the strain preserved in New York is descended from this, it corresponds with our W. This is the only 'Strong'

type we have received which has any claim to belong to the Flexner group. The 'Saigon' type from the Institut Pasteur, sent us in response to a request for a 'Strong', and also a strain received from St. Mary's Hospital, ferment saccharose but not mannite, give heavy acid agglutination and grow much more luxuriantly than Flexner's bacillus: they evidently belong to a different species. Now that we know that individual examples of various Flexner races may happen to ferment saccharose, it is no longer justifiable to apply the term 'Strong type' on that ground. We have avoided using the term altogether, and we gather that Major Strong himself lays no special stress on the type which has borne his name.

XI. EXAMINATION OF A MORE EXTENDED SERIES OF FLEXNER BACILLI.

Having satisfied ourselves as to the existence of the several serological races of Flexner's bacillus described in the preceding sections, and being furnished with plenty of rabbit sera peculiar to the different races, we proceeded to examine the long series of strains which had come into our hands. We used agglutination only for this purpose, as we found this nearly always sufficient to place a strain in its appropriate group, provided that regard is paid not so much to the absolute degree to which a serum agglutinates a strain, as to the percentage of its homologous titre to which the serum took the strain tested. We could thus neglect differences in titre amongst the sera employed.

The total number of strains examined, including those dealt with in the previous sections, was 116. We will now give the numbers of each race found, in order to give an idea of the relative commonness of each, and also, so far as we have the information, the geographical source, so as to convey some idea of their distribution. It must, however, be remembered that the assembling of troops in France from all parts of the world brought together on the Western front nearly all known types of dysentery bacilli.

The V Race.

Eleven of our strains were of pure V type, i. e. not agglutinated to any serious degree by Z sera. Five were from France, two from Salonica, one from asylum dysentery in England; in three the original source was unknown.

The VZ Sub-group.

Seventeen strains of primary V type were also agglutinated to a fair degree by Z sera, and are therefore presumed to belong to this sub-group. Thirteen were from France, and one each from Malta, Gallipoli, Mesopotamia, and Singapore.

The Z Race.

Twenty-one strains fell into this group, being agglutinated to titre or thereabouts by Z sera, and to a fairly high degree by X sera.

Fourteen were from France, two from England (one being an asylum dysentery), one came from Italy, one from Malta, and two from Singapore, while in one the source was unknown.

The X Race.

Eight strains appeared to belong to this race, being agglutinated by X sera only. Four were from France, two from Italy, one from Singapore, while one was sent from New York, its origin being unknown to us.

The WX Sub-group.

Twelve strains were agglutinated by X sera and also to a noteworthy degree by W sera, and hence are referred to this group. Seven were from France, two from England, one from Salonica, and one from Central Africa, while in one the source was unknown.

The W Race.

Twenty-nine of our strains were best agglutinated by a W serum, and are hence grouped as W's. They varied somewhat in the degree to which they were also agglutinated by V sera, some being almost untouched, others agglutinated to half titre. Of the 29, 16 were from France, two from English cases, two from Salonica, two from Malta, one from Italy, one from the United States, and one from Manila, while in four the place of origin was unknown.

The Y Race.

Eight of our strains appear definitely to belong to the Y group, being better agglutinated by one or other of our Y sera than by any others. Not one came from France. Four were from Italy, but in some of these infection may have occurred in Egypt. One was from Gallipoli and another probably from one of the Eastern fronts. One was from the United States and one from Manila. In two the place of origin was uncertain.

Doubtful Strains.

In 8 strains out of 116 examined (6·8 per cent.), agglutination alone left us in doubt as to the grouping: some of these were probably Y's, but as regards others we remain unsatisfied. It is, of course unlikely that our observations have covered all the serological varieties of Flexner's bacillus. Time was lacking for the adequate study of these aberrant strains, which would have demanded the preparation of sera and elaborate absorption tests. We must be content with having been able to effect a satisfactory grouping in 93 per cent. of the varied strains which have come into our hands.

Flexner Dysentery on the Western Front.

The relative proportions of the different races of Flexner's bacillus have probably varied in the several fields of war. Sixty-one of our strains came from cases infected in France and Belgium—rather more than half of the total we have examined. Our figures, therefore, probably give a fair idea of the relative proportions of

the different races prevalent on this front. Of these 61 strains, some 30 per cent. belonged to the *V* and *VZ* races, 23 per cent. to the *Z* race, 6.5 per cent. to the *X* race, 11.5 per cent. to the *WX* variety, 26 per cent. to the *W* race, while 3 per cent. could not be classed by agglutination alone. The true *Y* race appears to have been unrepresented on the Western front, although a considerable proportion of the cases in Italy during the 1918 epidemic seems to have been due to the *Y* bacillus, which also occurred with some freedom on certain of the Eastern fronts. Our strains from fronts other than the Western are, however, too few to allow of our forming any opinion as to relative prevalence of the different races there, though such strains as we have examined from Eastern sources seem to fall readily into the grouping we have put forward.

XII. PRACTICAL APPLICATION OF THE FACTS ELICITED.

When we commenced the work described in the preceding pages, it was the general experience in military and other laboratories that a considerable proportion of the bacilli of Flexner type isolated from cases of dysentery failed to show significant agglutination with the stock sera then available. Similarly, a great number of convalescents from dysentery of this type failed to show, in their serum, agglutination reactions with the stock Flexner and *Y* emulsions sent out from the standards laboratory at Oxford. These facts constituted the reason for attacking the problem, and they equally induced Captain Murray independently to carry on similar observations at the Millbank Laboratories. We have shown how the three sets of independent observations carried out by Gettings, Murray, and ourselves, are in reasonably complete harmony, and we believe that they will form the basis for a satisfactory solution of many of the serological problems connected with Flexner dysentery. We believe, too, that our results, and the methods by which they have been reached, are not without theoretical importance, inasmuch as they seem to offer one possible means for unravelling the antigenic constitution of the races of a serologically variable species. There are probably few species which offer a more favourable field for such an attempt than Flexner's bacillus, and although we do not feel that we have been completely successful, it seems possible that it is along such lines that problems of this kind may ultimately be solved.

Experiences with our Sera and Emulsions in France.

In August 1918, at a time when we believed we had defined the *V*, *Z*, *X*, and *W* races, but had not yet commenced the study of the *Y* races, one of us (A. C. I.) was directed to proceed to France in order to test the practical application of the knowledge gained. Meanwhile the other remained at St. Bartholomew's Hospital to pursue the study of the *Y* races.

In France, the practical application of the results was undertaken at No. 14 Stationary Hospital, where Major H. M. Perry, R.A.M.C., was in charge of the bacteriological laboratory. There was at this time a good deal of dysentery in the army, mostly of Flexner type.

The method of working which was adopted was as follows. When Major Perry isolated an organism of supposed Flexner type, he tested it with the Oxford Flexner and Y sera, which he had been in the habit of using for diagnosis. If the result proved negative he handed over the culture for more extended tests with our sera. Sixty-five cultures were thus examined. Three of them, on further examination, proved to be gas-formers, and were rejected. Twenty-two proved to be either late lactose-fermenters or the dulcitate-fermenting *B. alkalescens*. There remained forty strains, culturally of Flexner type, but not responding to the Oxford Flexner and Y sera. These were tested against a Z serum (Whittington), an X serum (Hughes), and a W serum (Cable). No less than thirty-four of the strains could be referred to our types. Owing to the fact that the X serum agglutinated the Z strains almost as well, in many cases, as the Z serum itself, it was not always easy to say from agglutination alone whether a strain was an X or a Z, especially as the X serum was one of higher titre than the Z serum: the strains well agglutinated with Z serum were classed as Z's, whether they were agglutinated by the X serum or not. On this basis twelve strains could be referred to the X and sixteen to the Z group. Five strains proved to belong to the W group, coming down with the Cable serum, though they had failed to do so with the Oxford Y serum. One strain was agglutinated by V, X, and Z sera, and appeared to belong to the V race. Six out of the forty strains could not be referred to any race with the sera available, which, it must be remembered, did not include any true Y serum. It is, however, a matter for satisfaction that 85 per cent. of the strains inagglutinable with the Oxford standard sera, could be recognized by the aid of the sera we had prepared.

A somewhat similar experience was met with later, in a visit to the Central American Laboratories at Dijon. Here a number of strains which had not responded to the American sera were similarly tested with sera prepared from the races defined by our work. Amongst them were seventeen strains culturally resembling Flexner's bacillus, and of these three could be referred to the V race, three to the Z, five to the X, one to the W, and one to the WX race; four strains only could not be determined, and subsequent investigation made it a little uncertain whether all of these were truly Flexner's bacillus.

It will thus be seen that the employment of V, Z, X, and W sera in routine laboratory diagnosis goes a long way to clear up the difficulties presented by the strains 'inagglutinable' by the sera in use in the earlier stages of the war. Such difficulties had, however, varied with the sera used, being less frequent where a serum such as the Lister Y was employed, owing to the fact that this serum presents marked group agglutinins for all but the X and W races. Even with this serum the race could not be determined.

Experience in Serodiagnosis.

Forty-three cases arriving at No. 14 Stationary Hospital within two days, all from a single outbreak, sent in as 'clinical dysentery'

(i. e. bacteriologically undiagnosed), were examined by serodiagnosis. The sera were tested against emulsions of Shiga's bacillus, and of four Flexner races, *V*, *Z*, *X*, and *W*, at a stage of the disease varying from the tenth to the thirty-second day from onset: six of the cases were examined twice. In 77 per cent. of the cases a positive result was obtained. In two cases the serum reacted with the Shiga emulsion, in nine cases with the *V*, in fourteen with the *Z*, and in eight with the *W* emulsion. In two of the *Z* cases there was also a much feebler reaction with the *X* emulsion. Ten cases were negative. The *V*, *Z*, and *W* emulsions were standardized ones issued from the Oxford Laboratories, so that the unitage of the sera could be determined. Of the *V* cases, four had less than 10 agglutinin units, and the highest had only 40. Of the *Z* cases, none were below 10 units, though two had only 11; the two highest had 88 and 225 units. Of the *W* cases the lowest had 18 units and the two highest 166 and 439 units respectively. The 'constants' assigned to the emulsions issued from the Oxford Laboratories are so adjusted as to render 10 units or more of probable significance in diagnosis; it will therefore be apparent that the majority of the results obtained in this series of cases yielded a presumptive diagnosis. The inclusion of the *Z* emulsion probably accounts for the proportion of positive results, which we believe to be considerably higher than has hitherto been obtained.

In another series of cases similarly examined, of which two proved to be *Z* cases and eight to be *W*'s, some curious facts were elicited as regards the *W* strains. It was discovered that in certain cases the velocity of the agglutination reaction was abnormally slow. After the customary 4 or 4½ hours in the water bath at 55° C., followed by 10 or 15 minutes' standing at room temperature, it sometimes happened that no agglutination could be detected; yet after standing at room temperature overnight, a fairly high titre for *W* might be manifest in the patient's serum, even up to 100 or more agglutinin units. This phenomenon was observed in the case of no other Flexner race, and it renders it needful always to allow this prolonged period of standing before reading the results when testing a serum against a *W* emulsion. It was seen chiefly with the Oxford *Y* emulsion, but to some extent with a Cable emulsion also.

Another singular point was noted in this series of eight *W* cases. The serum was tested against both Oxford *Y* and Cable emulsions. Three of the cases yielded a marked positive result with the Oxford *Y*, but were quite negative to the Cable emulsion. The remaining five were positive to both, but all of them showed a higher titre with the Oxford *Y* emulsion. We have studied both these strains carefully, and they are not quite alike serologically, though we group them both as *W*'s: certain *W* strains have a considerable *V* element in their constitution, in others it seems almost absent; the Oxford *Y* belongs to the former, Cable to the latter group. This may in part account for the above fact, but it may also depend on difference in agglutinability between the two strains; the Oxford *Y* was selected on account of its high agglutinability, but the Cable strain is unstandardized in this respect.

Observations were also made on the serum of a number of cases

already diagnosed by the recovery of bacilli of different types from the stools. In 25 cases the *V* type had been isolated. In 23 of these the patients' serum agglutinated the standard Oxford Flexner emulsion (6 to 80 units). In 22 of the cases the serum was tested against the bacillus from the patients' own stools: a positive reaction was observed in 18 cases.

Of five cases from which *Z* strains had been isolated, the patients' serum agglutinated the standard Oxford *Z* emulsion in four. In three out of six such cases the serum agglutinated the patients' own bacillus.

In two cases from which *X* strains had been isolated the serum of both reacted with the patients' own bacillus. In one case the serum agglutinated emulsions of *V*, *Z*, and *X* type, thus reproducing, in the human being, the wide agglutinative range of rabbit serum prepared with the *X* race.

Of four cases from which *W* strains had been isolated, the patients' serum agglutinated both the Oxford *Y* emulsion and the patients' own organism in two instances, and in a third agglutinated the latter only.

The preceding facts suggest that when the laboratory worker is provided with a suitable range of agglutinable emulsions, embracing the various types we have described, serodiagnosis will become a more successful means of recognizing Flexner dysenteries than it has been in the past, even if it does not exhibit the uniform success which attends the method in Shiga cases.

Further Observations on the Agglutinative Range of an X Serum.

The richness of a rabbit serum prepared from an *X* race in group agglutinins for other Flexner races (except *W*) renders it an exceedingly useful monovalent serum for general use, as is seen in the following observations. Seventy-one different Flexner strains were put up with an *X* serum, and agglutination was noted in no less than 83 per cent. The results are shown in the following table:

<i>Serological race.</i>	<i>Number examined.</i>	<i>Positive.</i>	<i>Negative.</i>
<i>V</i>	26	26	0
<i>Z</i>	22	20	2
<i>X</i>	12	12	0
<i>W</i>	11	1	10

It is seen that almost the only negative results are with strains of the *W* race.

In concluding this account of our work on the serological classification of the Flexner group of bacilli we wish to make it clear that we are fully conscious of the difficulty of the problem. We have endeavoured to attack it by the use of strictly quantitative methods, employing the technique which we believed to be the best adapted for the purpose. The data obtained have been set out fully and without any reserve. We have stated the conclusions which appeared to us to be justified by our data, but we recognize that such conclusions must be subject to revision in the light of further knowledge. In particular, we feel that further facts are required in

relation to the antigenic structure of the Y races of Flexner's bacillus. We have met, moreover, with a certain small residuum of strains which we cannot exclude from the Flexner group, but which do not fit in with the classification we have propounded. The recent war has presented remarkable opportunities for the study of dysentery bacilli, but it is unlikely that our material has covered the whole field.

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EXPLANATION OF DIAGRAMS.

The principles upon which these diagrams are constructed have been set forth in the body of the report (pp. 17, 18, 19 and 25).

They are in all cases *percentage* diagrams. In the case of a serum, the titre for the homologous bacillus is stated as 100, and the titres for other strains as proportions of this. In the case of a bacillus, the height of each column represents the percentage of the titres of the various sera for their homologous bacilli, to which it is agglutinated. In the absorption diagrams the curves similarly express percentages of the titres for the different strains tested, the titre for each in the unabsorbed serum being taken as 100.

The colours are uniform throughout, and have the following significance.

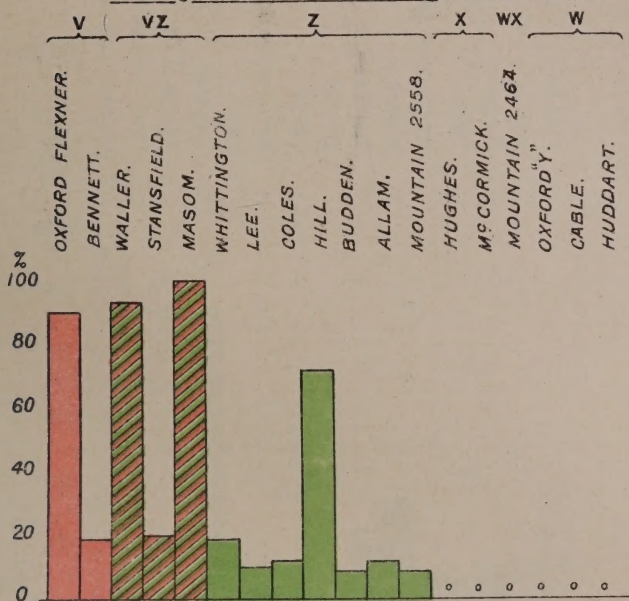
- Red = V strain, serum or bacillus as the case may be.
- Green = Z strain.
- Blue = X strain.
- Black = W strain.
- Dotted black = Y strain.

In the 'spectra' of sera and bacilli, VZ strains are represented as red hatched with green, and WX strains as blue hatched with black.

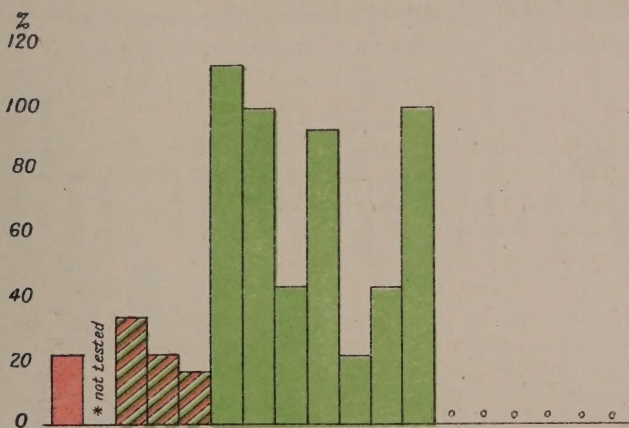
Fig. 1.

PERCENTAGE TITRES OF 3 HUMAN SERA FOR 18 STRAINS OF FLEXNERS BACILLUS.

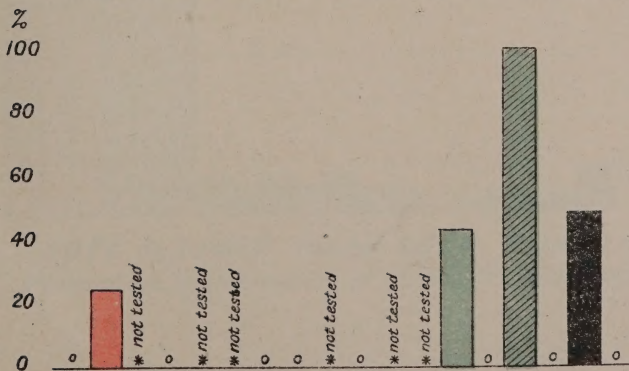
Showing indications of serological grouping.



Human Serum MASOM (Titre for its own bacillus = 177) VZ race



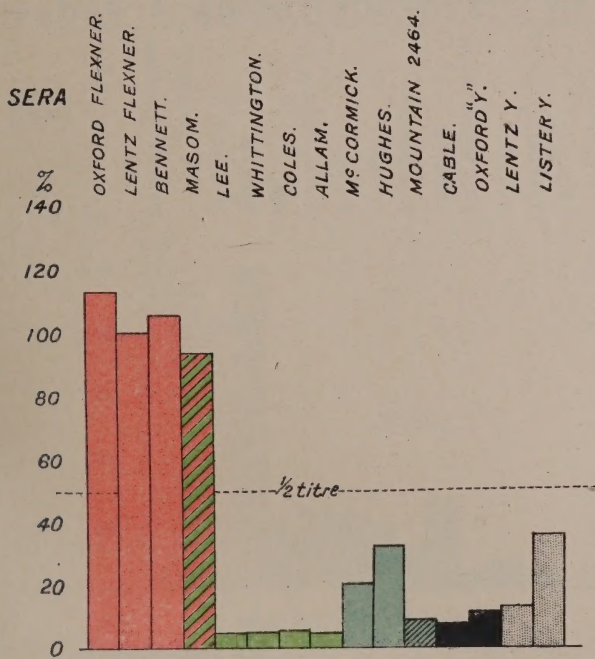
Human Serum MOUNTAIN 2558 (Titre for its own bacillus = 75) Z race



Human Serum MOUNTAIN 2464 (Titre for its own bacillus = 67) WX race

Fig. II.

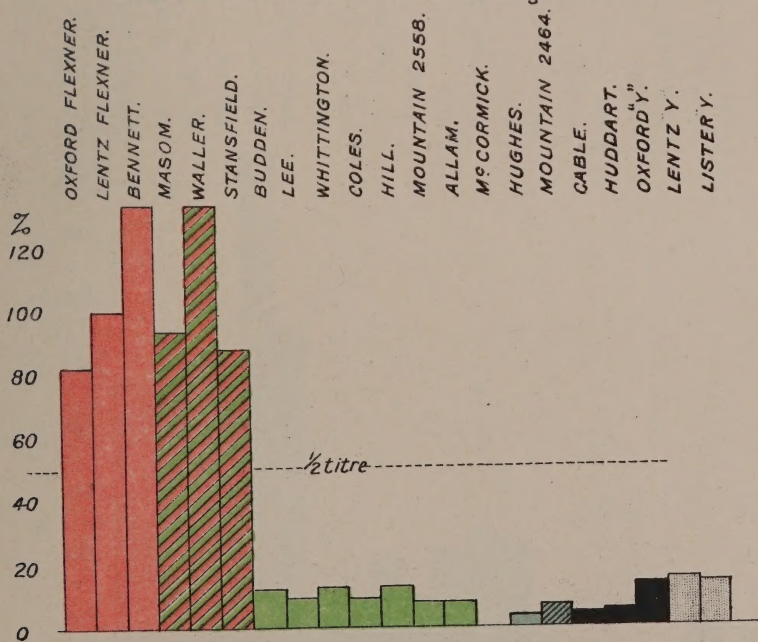
AGGLUTINATION & AGGLUTINOGENESIS OF A V STRAIN.



LENTZ FLEXNER Bacillus

Percentage agglutination by 15 monovalent rabbit sera

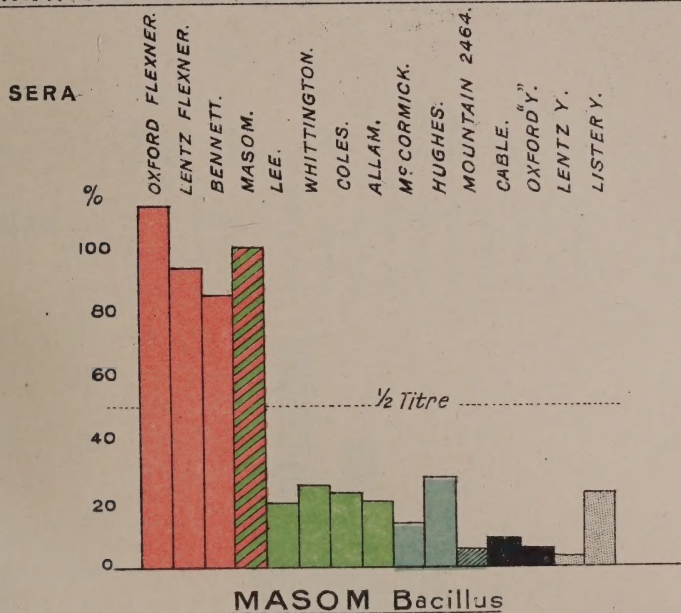
Titre of each serum for its homologous strain = 100



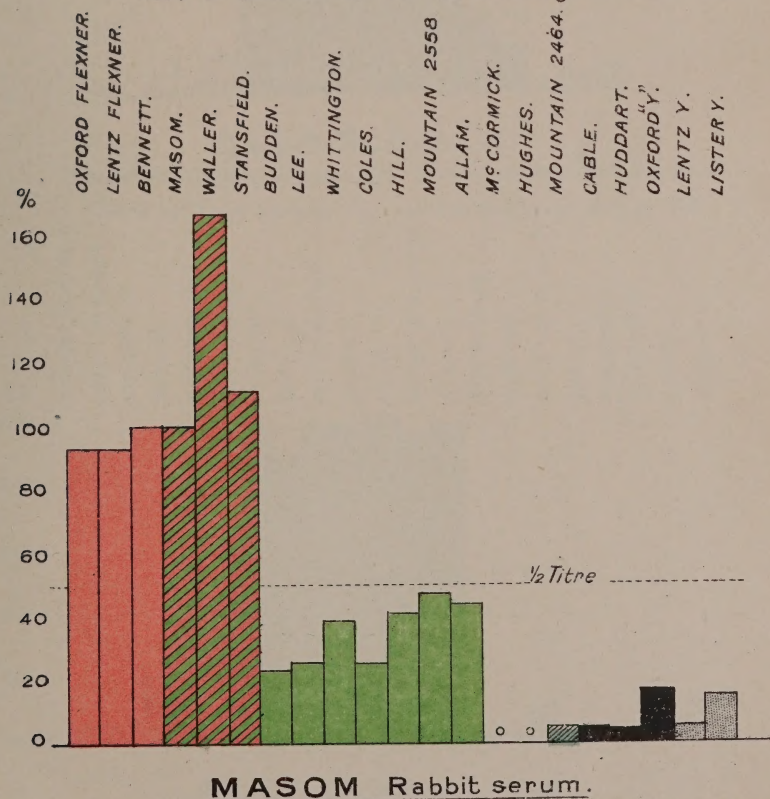
LENTZ FLEXNER Rabbit Serum. Titre for itself = 1881
Percentage agglutination of 21 bacilli (homologous = 100)

Fig.III.

AGGLUTINATION & AGGLUTINOGENESIS OF A VZ STRAIN.



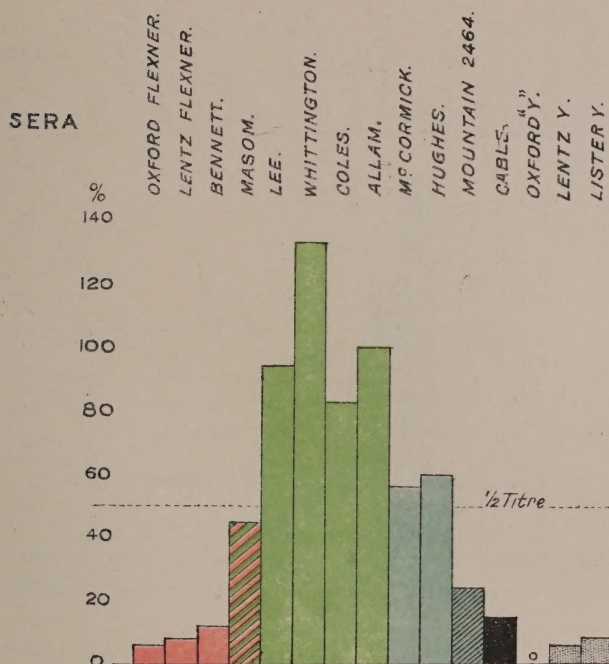
Percentage agglutination by 15 monovalent rabbit sera.
 Titre of each serum for its homologous strain=100.



Percentage agglutination of 21 bacilli. (homologous=100)
 Titre for itself=747.

Fig. IV.

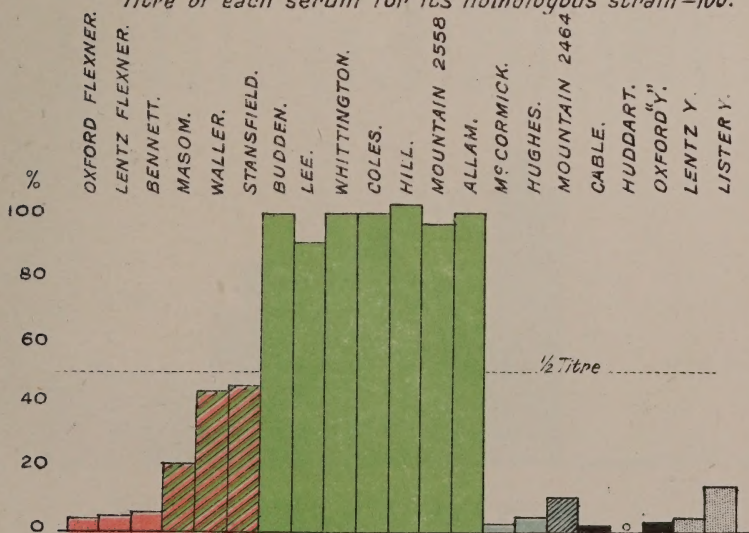
AGGLUTINATION & AGGLUTINOGENESIS OF A Z STRAIN.



ALLAM Bacillus.

Percentage agglutination by 15 monovalent rabbit sera.

Titre of each serum for its homologous strain = 100.



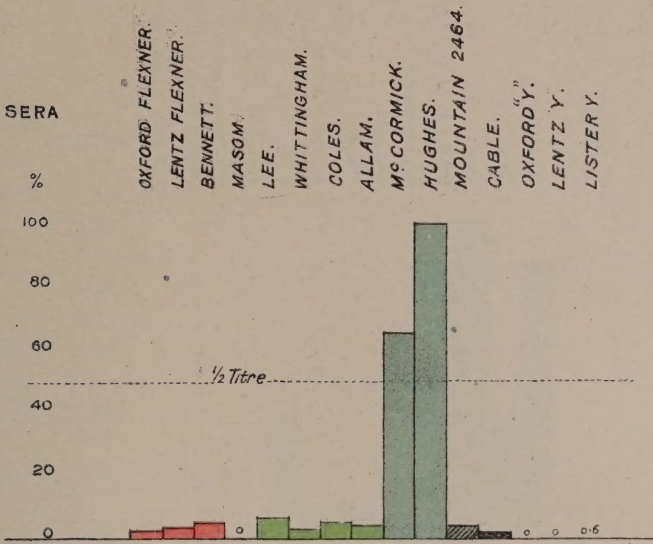
ALLAM Rabbit serum.

Percentage agglutination of 21 bacilli. (homologous = 100.)

Titre for itself = 1606.

Fig. V.

AGGLUTINATION & AGGLUTINOGENESIS OF AN X STRAIN.



HUGHES Bacillus.

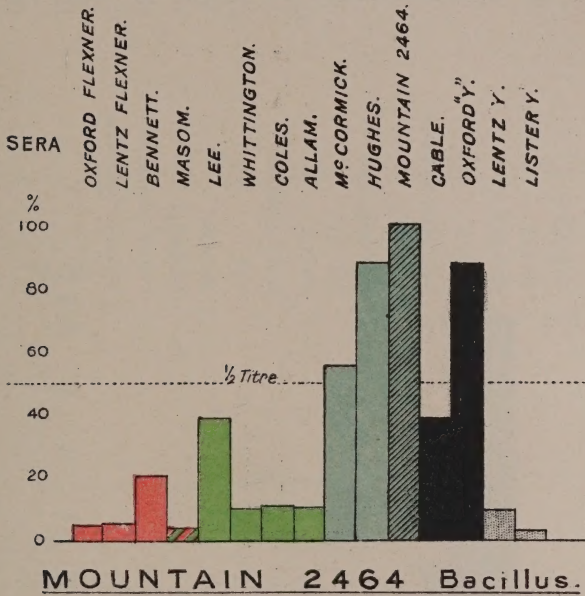
Percentage agglutination by 15 monovalent rabbit sera.
 Titre of each serum for its homologous strain=100.



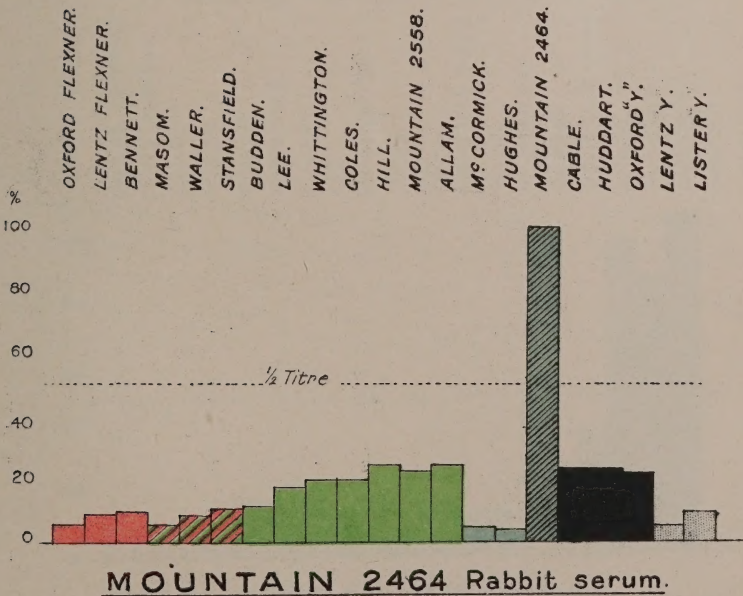
HUGHES Rabbit serum

Percentage agglutination of 21 bacilli (homologous=100)
 Titre for itself=2500.

Fig. VI.

AGGLUTINATION & AGGLUTINOGENESIS OF A WX STRAIN.

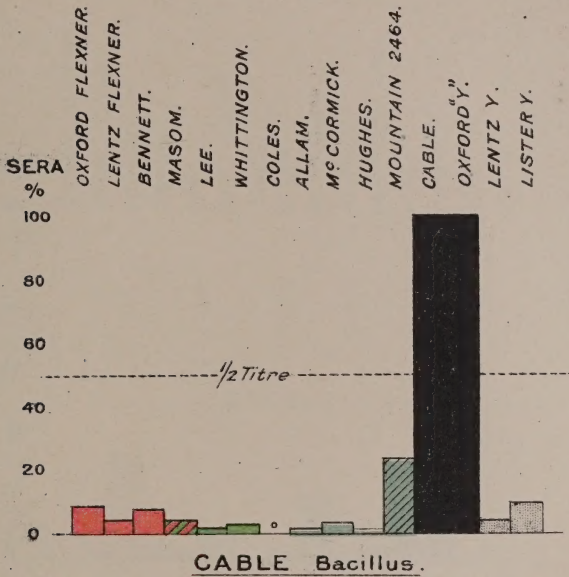
Percentage agglutination by 15 monovalent rabbit sera.
 Titre of each serum for its homologous strain=100.



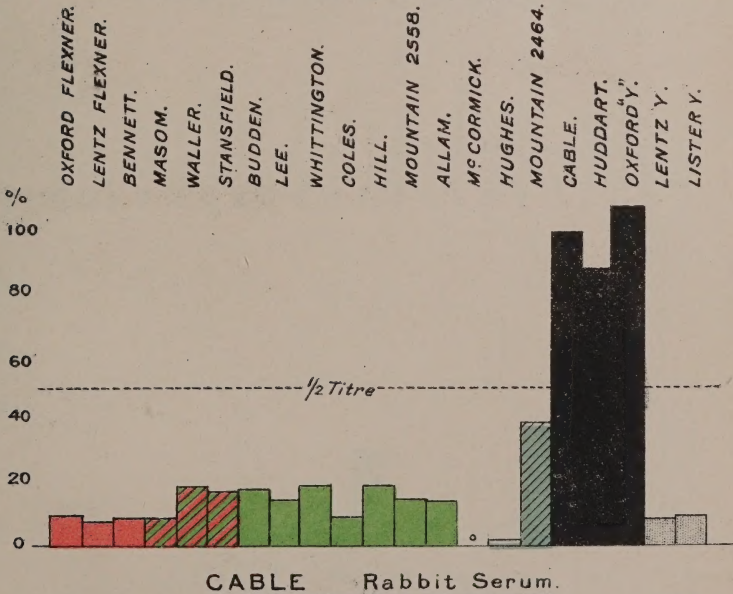
Percentage agglutination of 21 bacilli. (homologous=100)
 Titre for itself=1456.

FIG. VII.

AGGLUTINATION & AGGLUTINOGENESIS OF A W STRAIN



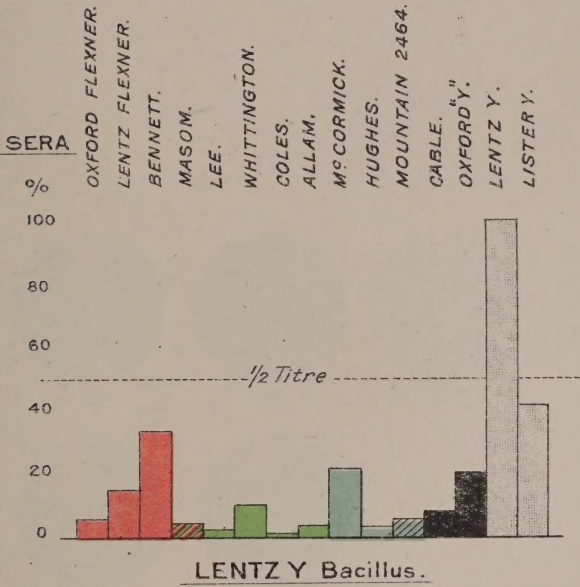
Percentage agglutination by 15 monovalent rabbit sera.
 Titre of each serum for its homologous strain = 100.



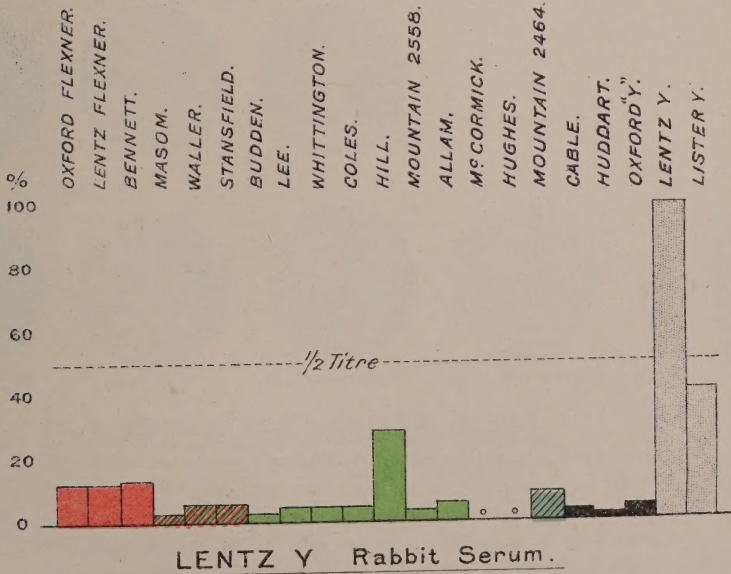
Percentage agglutination of 21 bacilli (homologous = 100)
 Titre for itself = 848.

FIG. VIII.

AGGLUTINATION & AGGLUTINOGENESIS OF A Y STRAIN.



Percentage agglutination by 15 monovalent rabbit sera.
Titre of each serum for its homologous strain = 100.



Percentage agglutination of 21 bacilli (homologous = 100)
Titre for itself = 2675.

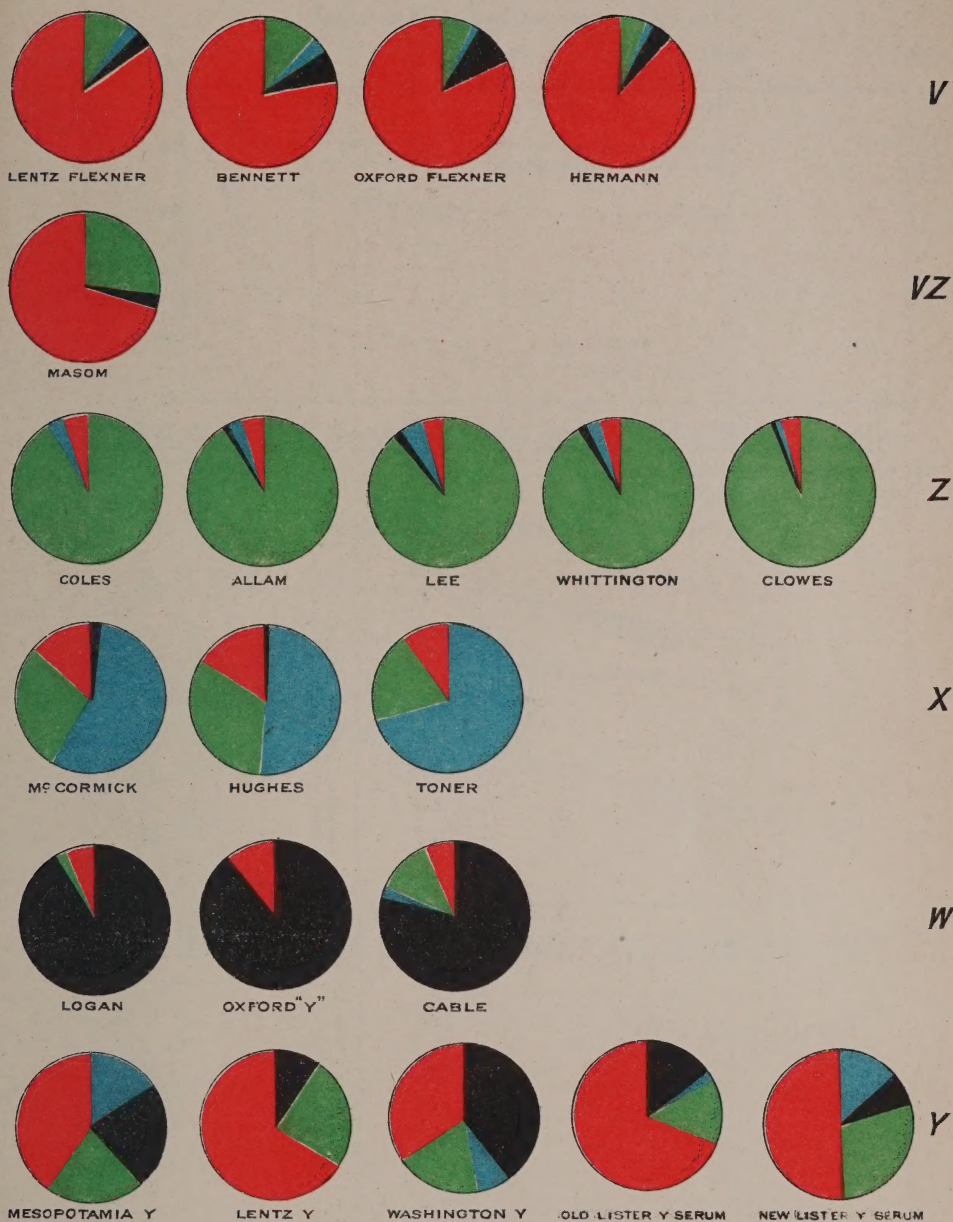
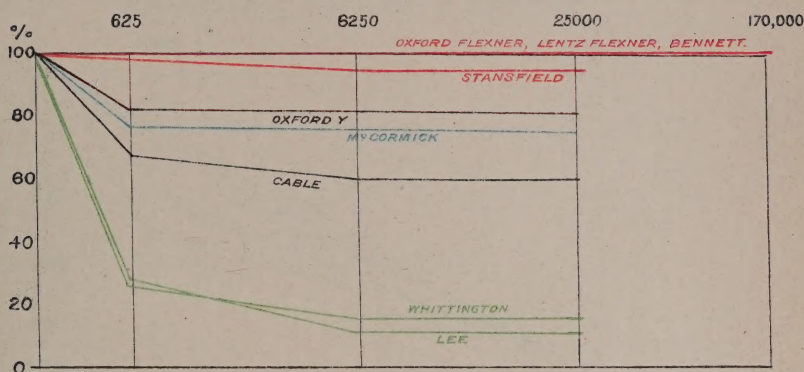


FIG. IX. ANALYSIS OF 21 FLEXNER SERA.

The circles represent the relative agglutinating powers upon V, Z, X and W strains calculated from our data for 21 Flexner Sera. Only 4 antigenic components are assumed. In so far as antigenic structure is revealed by agglutinogenic power, the sectors may suggest this structure. In the case of the V, VZ, Z and W races it is possible that the proportions of the different components shown are not far from the truth. Much more doubt attaches to the X and Y strains.

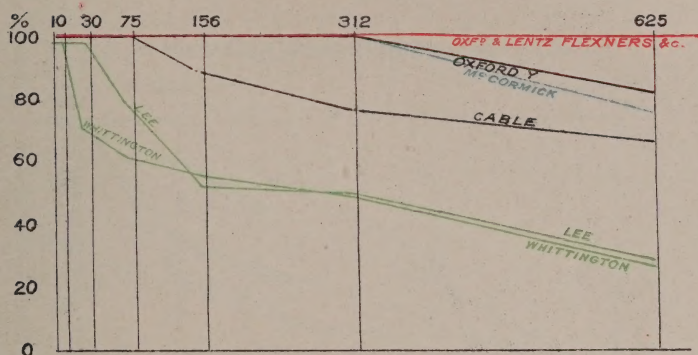
Absorbing doses in millions per c.c. Serum.



OXFORD FLEXNER Serum absorbed with WHITTINGTON bacillus.

Per-centage reductions of titre for the 9 strains indicated.

Absorbing doses in millions per c.c. Serum.

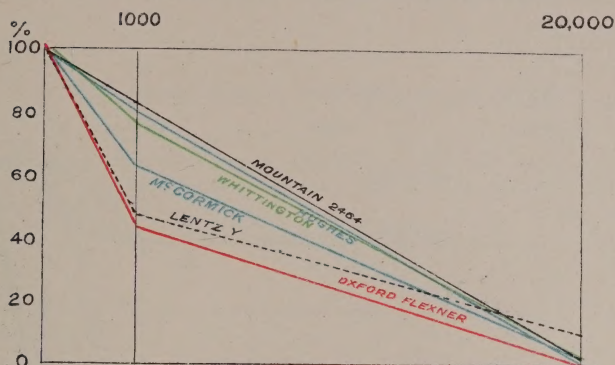


EARLIER STAGES of same absorption, -drawn to scale.

FIG. X. Absorption of a V Serum with a Z Bacillus.

*The upper figure shows the ordinary form of absorption exp.^d -
The lower figure shows the effect of very minute absorbing
doses upon the secondary Z agglutinin in the V serum.
The primary V agglutinin is not absorbed.*

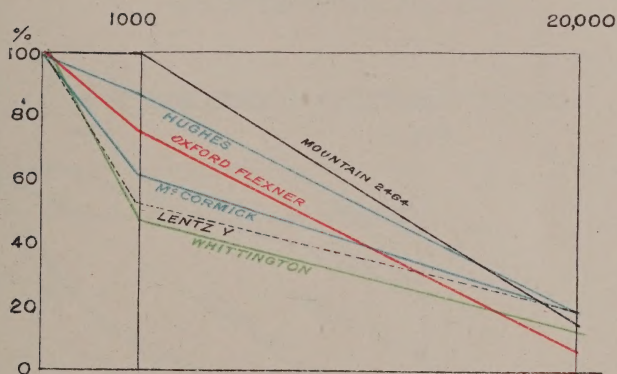
Absorbing doses in millions per c.c. Serum.



HUGHES Serum absorbed with M^c CORMICK bacillus.

*Per-centage reductions in titre
for the 6 strains indicated.*

Absorbing doses in millions per c.c. Serum.



M^c CORMICK Serum absorbed with HUGHES bacillus.

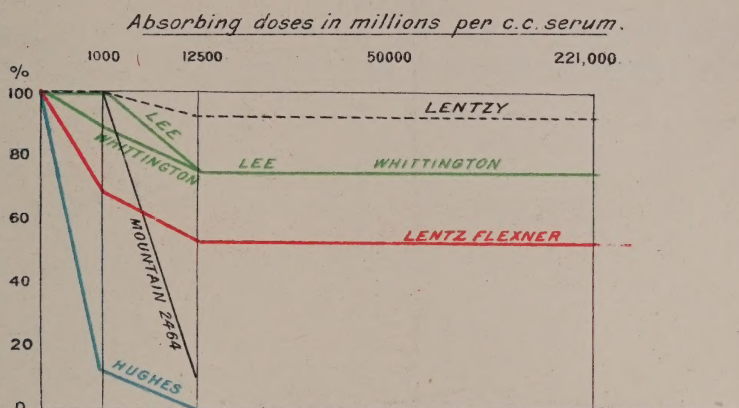
*Per-centage reductions in titre
for the 6 strains indicated.*

FIG. XI. Reciprocal absorptions between two strains of identical race. (race X).

Each behaves to the other exactly as if it were the homologous organism, all agglutinins, primary and secondary, being alike almost absorbed. This constitutes proof of racial identity.

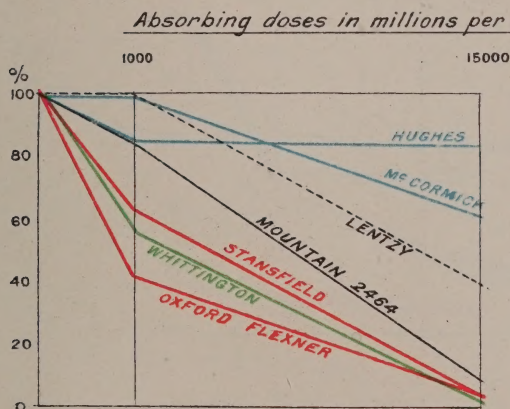
FIG XII.

RECIPROCAL ABSORPTIONS BETWEEN Z & X STRAINS.



Z Serum LEE Absorbed with X Bacillus HUGHES.

% Reductions in Titre.



X Serum HUGHES Absorbed with Z Bacillus WHITTINGTON.

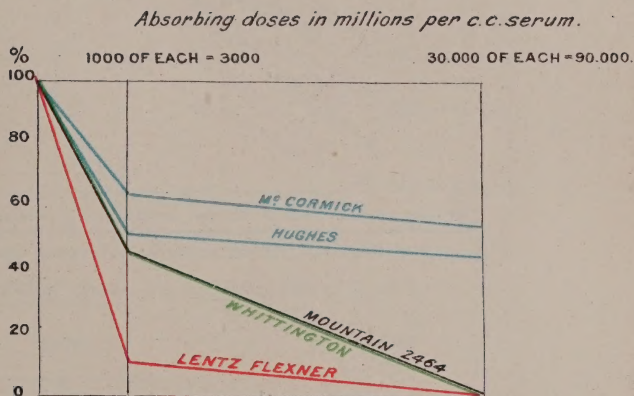
% Reductions in Titre.

There is a slight degree of mutual absorption, but even an enormous dose of X antigen only reduces the Z agglutinin by 25%. A moderate dose of Z antigen reduces the X agglutinin by 15% in the case of the homologous bacillus & by 37% in the case of M^c Cormick.

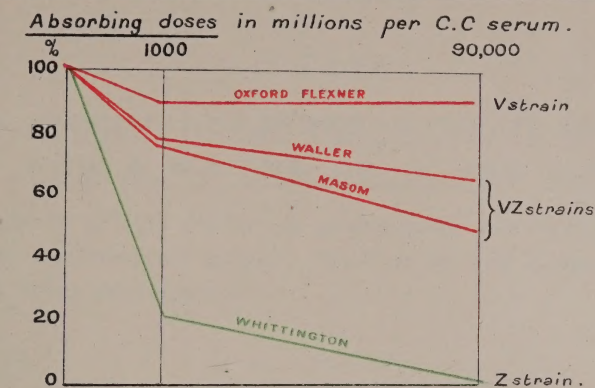
The two strains are essentially heterologous, but not completely so; probably owing to a secondary Z antigen in Hughes (shown by its agglutinogenic powers.)

FIG XIII.

MULTIPLE ABSORPTION OF AN X SERUM-HUGHES.
WITH EQUAL PARTS OF V.Z & W STRAINS SIMULTANEOUSLY.



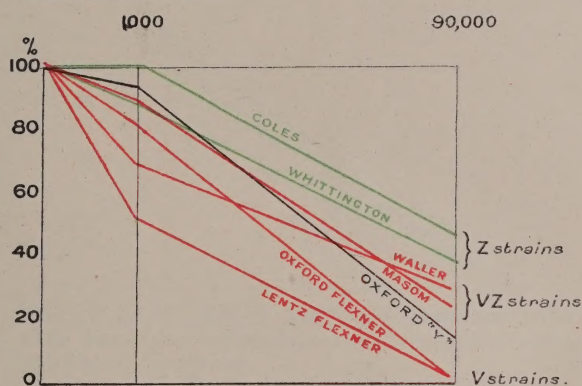
*Shewing percentage reductions in Titre for the 5 strains indicated.
 The combination of large doses of the three other antigens
 fails to remove more than about half the agglutinin for the
 two X strains.*



VZ serum absorbed with Z bacillus alone.

Percentage reductions in titre for the 4 strains indicated

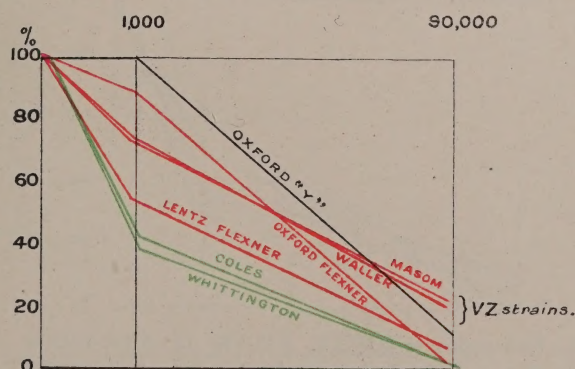
Absorbing doses in millions per C.C serum.



VZ serum absorbed with V bacillus alone.

Percentage reductions in titre for the 7 strains indicated

Absorbing doses in millions per C.C serum.



VZ serum absorbed with a combination of equal parts of V & Z bacilli.

Percentage reductions in titre for the 7 strains indicated.

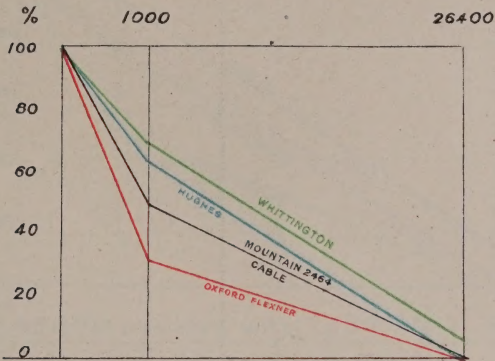
Fig. XIV. Absorptions of a VZ serum (MASOM) with a V strain (Oxford Flexner) & a Z strain (Whittington) separately & in combination. VZ is more heterologous with Z than with V: the mixture of the two effects an absorption almost homologous in type. In the two upper figures the VZ agglutinins behave in a manner intermediate between those for V and those for Z.

Fig.XV.

A W.X. SERUM ABSORBED WITH ITS OWN BACILLUS
AND WITH A MIXTURE OF W. AND X. STRAINS.

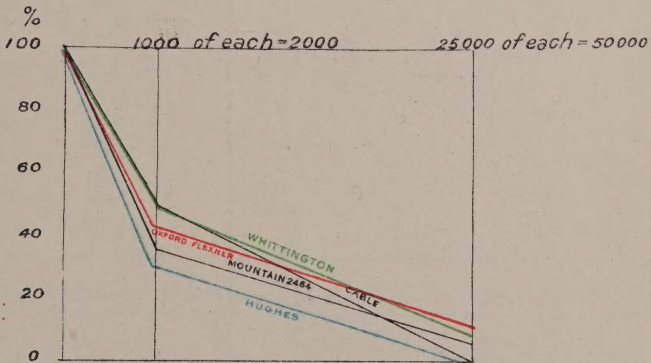
The effect of the latter is practically identical with that of the homologous strain. Neither W. nor X.alone is able to effect this absorption.

Absorbing doses in millions per c.c.serum.



MOUNTAIN 2464 SERUM absorbed with its own bacillus. Percentage reductions in titre for the 5 strains indicated.

Absorbing doses in millions per c.c.serum.



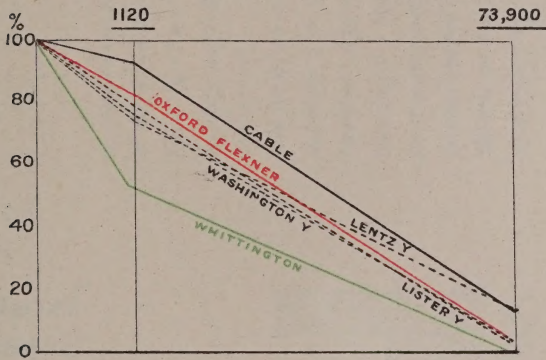
MOUNTAIN 2464 SERUM absorbed with equal parts of CABLE (W) and HUGHES (X) bacilli. Percentage reductions in titre for the 5 strains indicated.

Fig.XVI.

RECIPROCAL ABSORPTIONS BETWEEN THE LENTZ Y and LISTER Y STRAINS

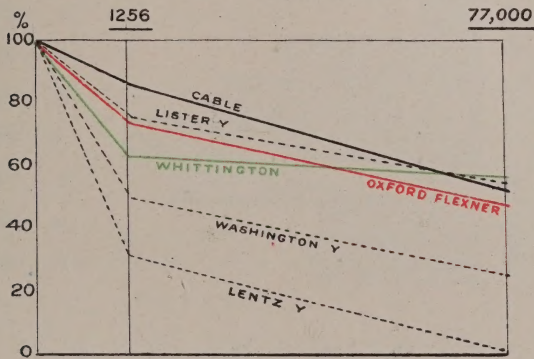
Shewing percentage reductions of titre for the 6 strains indicated.

Absorbing doses in millions per C.C serum.



LENTZ Y SERUM absorbed with LISTER Y BACILLUS.

Absorbing doses in millions per C.C serum.



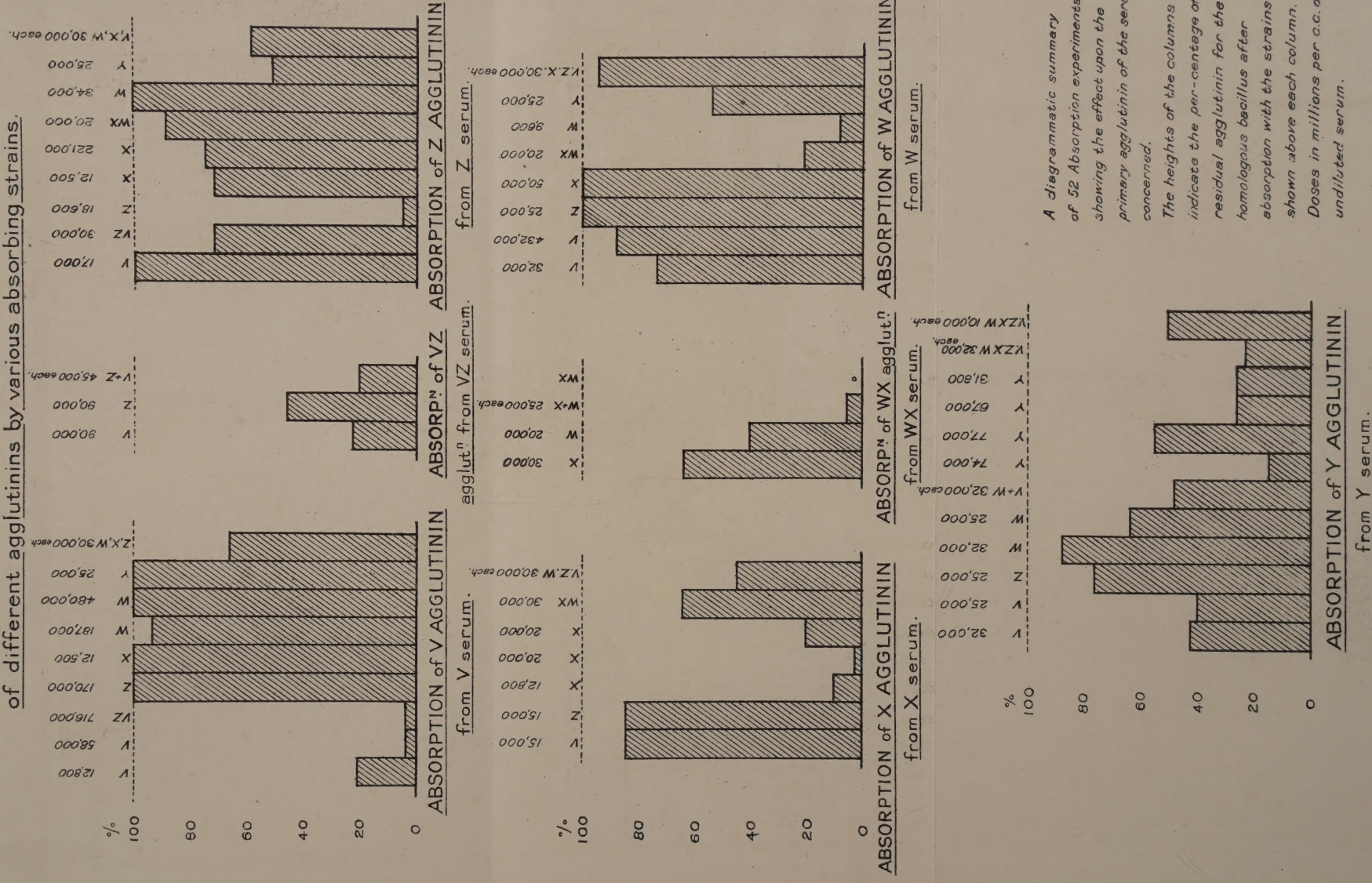
LISTER Y SERUM absorbed with LENTZ Y BACILLUS.

The Lister Y bacillus exhausts the Lentz Y serum almost completely, the absorption being of the homologous type.

The Lentz Y serum effects only incomplete exhaustion of the Lister Y serum. Only half the primary agglutinin, and half of the secondary agglutinins for V and Z are removed.

FIG. XVII.

PER-CENTAGE ABSORPTIONS of different agglutinins by various absorbing strains.



A diagrammatic summary of 52 Absorption experiments, showing the effect upon the primary agglutinin of the serum concerned.

The heights of the columns indicate the per-centage of residual agglutinin for the homologous bacillus after absorption with the strains shown above each column. Doses in millions per c.c. of undiluted serum.

APPENDIX

THE tables which follow contain the data of the experiments on which the preceding report is based. The figures are given as revealed by the agglutinations, or as calculated by Dreyer's reduction table, and are stated as plain numerals to save space. The figure 2,500 means standard agglutination in a serum dilution of 1 in 2,500. As regards the apparent pedantry in the exactness with which the figures are stated, see remarks on p. 16 of the preceding report.

1. CROSS-AGGLUTINATIONS OF EIGHTEEN STRAINS OF FLEXNER BACILLI WITH TWELVE HUMAN SERA FROM CASES OF CONVALESCENT DYSENTERY. Actual titres. [0 means a negative result. Dots mean that the test was not carried out.]

BACILLI.		SERA.										X and WX strains.		
		V and VZ strains.			Z strains.									
		Bennett.	Waller.	Masom.	Whittington.	Lee.	Coles.	Hill.	Allam.	Mountain 2,558	Hughes.	McCormick.	Mountain 2,464	
V	Oxford Flexner .	80	26	160	0	27	0	35	20	17	29	50	0	
	Bennett .	367	44	34	33	31	17	..	26	..	17	75	17	
VZ	Waller .	..	31	165	..	31	..	..	..	26	..	125	..	
„	Stansfield .	31	31	35	17	17	0	33	27	17	0	147	0	
„	Masom .	40	20	177	..	31	..	..	17	13	..	76	..	
Z	Whittington .	43	44	33	36	33	..	73	91	85	0	64	..	
„	Lee .	29	35	19	37	34	17	50	74	75	0	75	0	
„	Coles .	35	33	22	36	31	20	71	79	33	0	79	0	
„	Hill .	..	50	128	..	67	..	155	91	70	..	125	..	
„	Budden .	29	22	17	35	27	0	35	69	17	0	75	0	
„	Allam .	29	35	22	..	33	..	..	38	33	..	80	..	
„	Mountain 2,558 .	31	22	17	30	31	..	44	72	75	..	50	..	
X	Hughes .	0	0	0	0	0	0	..	0	0	13	17	29	
„	McCormick .	0	0	0	0	0	..	..	26	0	0	40	0	
WX	Mountain 2,464 .	0	17	0	0	0	0	0	20	0	0	19	67	
W	Oxford Y .	75	0	0	0	0	0	0	0	0	36	19	0	
„	Cable .	0	0	0	0	0	0	32	0	0	29	17	33	
„	Huddart .	0	0	0	0	17	0	31	0	0	29	32	0	

2. CROSS-AGGLUTINATIONS OF TWENTY-ONE FLEXNER STRAINS WITH MONOVALENT RABBIT SERA PREPARED FROM FIFTEEN OF THEIR NUMBER. Actual titres. (Two of the sera, Oxford Flexner and Oxford Y, were old ones; the remainder had been recently prepared. The Lister Y serum was an old one from the Lister Institute.)

	SERA.														
	V-strains.			VZ.	Z-strains.			X-strains.		WX.	W-strains.		Y-strains.		
	Oxford Flexner.	Lentz Flexner.	Bennett.	Masom.	Lee.	Whittington.	Coles.	Allam.	McCormick.	Hughes.	Mountain 2,469	Cable.	Oxford Y.	Lentz Y.	Lister Y.
<i>Bacilli.</i>															
V Oxford Flexner	1,456	1,556	1,100	697	74	69	125	69	321	653	80	83	302	331	2,500
" Lentz Flexner	1,656	1,881	1,556	697	79	69	165	80	250	803	125	64	282	331	2,200
" Bennett	2,500	2,500	1,456	747	84	74	165	110	314	742	145	69	322	353	2,500
VZ Masom	1,656	1,768	1,250	747	353	331	697	331	165	697	80	72	165	75	1,368
" Waller	1,656	2,500	1,250	1,250	500	564	1,606	707	155	687	125	155	220	165	1,100
" Stansfield	1,656	1,656	1,250	831	697	707	1,606	747	250	1,250	155	145	250	165	880
Z Budden	110	220	282	176	1,556	677	2,200	1,606	250	1,656	170	150	0	71	—
" Lee	125	165	165	194	1,763	742	2,500	1,456	331	1,506	250	125	0	125	353
" Whittington	125	250	250	291	2,500	1,250	3,025	1,606	500	1,557	291	165	17	125	697
" Coles	137	176	250	194	1,763	742	3,025	1,606	625	1,763	291	84	0	125	353
" Hill	125	250	282	311	2,500	1,656	3,225	1,656	703	1,831	353	165	0	747	697
" Mountain 2,558	80	155	250	353	1,763	1,506	2,825	1,557	703	1,557	331	128	0	88	500
" Allam	84	155	165	331	1,656	1,656	2,500	1,606	703	1,506	353	125	0	160	500
X McCormick	30	0	0	0	61	44	79	30	1,250	1,125	74	0	0	0	50
" Hughes	32	60	69	0	125	33	155	69	848	2,500	65	15	0	0	37
WX Mountain 2,464	87	125	302	33	697	141	331	165	697	2,200	1,456	331	2,200	250	170
W Cable	125	74	110	33	29	37	0	17	30	15	331	848	2,500	75	565
" Huddart	155	84	125	26	15	33	0	0	31	17	331	747	3,025	37	440
" Oxford Y	322	250	176	125	65	33	0	35	29	29	311	917	2,500	125	1,768
Y Lentz Y	84	282	500	33	50	125	29	69	275	80	84	69	500	2,625	2,500
" Lister Y	311	250	440	110	176	311	321	220	697	165	141	80	697	1,125	6,050

Note.—The homologous titres are in heavy type. The potency of the individual sera is seen to vary greatly, and there were probably considerable differences in the agglutinability of the different bacillary emulsions of the same race. Compare the second table, Appendix : 14.

3. HOMOLOGOUS ABSORPTIONS OF V SERA.

- (a) *Absorption of Oxford Flexner serum* with four different doses (small to moderate) of the *Oxford Flexner bacillus*. Shows reduction in titre for all strains tested, heterologous as well as homologous; the absorption has not been pushed to the extinction of the homologous agglutinin. Serum dilution 1 in 10.

Strains against which serum was tested.		Un- absorbed control.	Absorbing doses per c. c. serum.			
			200 million.	800 million.	3,200 million.	12,800 million.
V	Oxford Flexner	3,312	3,312	3,312	3,025	697
VZ	Stansfield	6,450	6,050	5,000	1,656	747
"	Waller	5,654	5,650	5,000	3,318	747
"	Masom	6,050	5,650	5,000	2,825	747
Z	Allam	331	311	250	165	33
"	Mountain 2,558	331	301	250	165	28
"	Budden	125	120	120	74	19
"	Coles	165	155	155	125	29
"	Lee	165	155	155	110	17
"	Whittington	250	250	250	165	28
W	Oxford Y	747	697	610	282	74
"	Cable	500	500	353	165	33
"	Huddart	500	500	440	165	28

- (b) *Absorption of Lentz Flexner serum* with a small and a large dose of a VZ strain (*Cahourg*). Shows virtual extinction of agglutinins for all strains tested. Serum dilution 1 in 10.

Strains against which serum was tested.		Unabsorbed control.	Absorbing doses per c. c. serum.	
			1,236 million.	58,212 million
V	Lentz Flexner	1,132	824	33
"	Oxford Flexner	1,132	720	31
VZ	Cahourg	2,120	1,016	59
Z	Whittington	125	50	0
W	Cable	233	125	0

- (c) *Absorption of Oxford Flexners erum* with four different doses (large to enormous) of a VZ strain (*Masom*). Shows final extinction of agglutinins for all strains tested, except residual traces for V strains. Serum dilution 1 in 50.

Strains against which serum was tested.		Un- absorbed control.	Absorbing doses per c. c. serum.			
			89,500 million.	179,000 million.	358,000 million.	716,000 million.
V	Oxford Flexner	3,112	828	401	337	140
"	Bennett	5,000	778	391	158	85
VZ	Masom	3,112	373	147	85	0
"	Stansfield	3,662	382	137	85	0
Z	Allam	170	0	0	0	0
"	Budden	170	0	0	0	0
"	Coles	170	0	0	0	0
"	Mountain 2,558	170	0	0	0	0
W	Oxford Y	675	66	0	0	0
"	Cable	230	0	0	0	0
"	Huddart	205	0	0	0	0

4. HETEROLOGOUS ABSORPTIONS OF V SERA.

- (a) *Absorption of Oxford Flexner serum* with nine different doses of a *Z bacillus* (*Whittington*), commencing with very small doses. The effect upon the titre for the absorbing strain begins at a dose of 30 million. The *V* agglutinin is unaffected even by the largest dose. Serum dilution 1 in 10.

[NOTE:—This table is a composite one embodying three separate experiments. The control titres were not always the same, and the results have been calculated so as to render them comparable.]

		Absorbing doses per c. c. serum.									
Strains against which serum was tested.		Unabsorbed control.	10 mil. lion.	30 mil. lion.	75 mil. lion.	156 mil. lion.	312 mil. lion.	625 mil. lion.	6,250 mil. lion.	25,000 mil. lion.	170,000 mil. lion.
V	Oxford Flexner	3,312	..	..	..	..	..	..	3,312	3,312	3,312
„	Lentz Flexner	7,925	..	..	..	..	..	..	7,925	7,925	7,925
„	Bennett	7,925	..	..	..	..	..	..	7,925	7,925	7,925
VZ	Stansfield	7,925	..	..	..	..	..	..	7,475	7,475	..
Z	Whittington	500	500	352	310	283	249	118	80	80	..
„	Lee	625	625	625	518	329	311	179	70	70	..
X	McCormick	165	..	..	..	165	165	125	..	..	..
W	Oxford Y	747	..	..	..	747	747	613	605	605	..
„	Cable	747	..	..	747	653	565	500	445	445	..

- (b) *Lentz Flexner serum* absorbed with a small and a moderate dose of an *X strain* (*Hughes*). The small titre for *X* quickly disappears, and that for a *WX* strain is much reduced. The *V* agglutinin remains untouched. Serum dilution 1 in 10.

		Absorbing doses per c. c. serum.		
Strains against which serum was tested.		Unabsorbed control.	1,000 million.	12,500 million.
V	Lentz Flexner	1,763	1,763	1,763
Z	Lee	155	137	137
„	Whittington	176	145	145
X	Hughes	68	0	0
WX	Mountain 2,464	110	37	37
Y	Lentz Y	282	250	250

- (c) *Oxford Flexner serum* absorbed with three small and moderate doses of a *W strain* (*Oxford Y*), and, in another experiment, with a very large dose of the same strain. The *V* agglutinin is only absorbed to a trivial extent. Serum dilution 1 in 10, and with the big dose 1 in 100.

		Absorbing doses per c. c. serum.				
Strains against which serum was tested.		Un- absorbed control.	1,170 million.	4,680 million.	18,720 million.	187,200 million.
V	Oxford Flexner .	3,762	3,312	3,317	3,317	3,317
„	Lentz Flexner .	11,000	10,250	..	..	..
VZ	Masom .	3,112	3,112	3,112	..	..
Z	Allam .	165	165	165	..	..
„	Mountain 2,558	150	155	..	..	..
W	Oxford Y .	1,125	570	250	37	0
„	Cable .	220	155	72	..	..
„	Huddart .	220	155	..	..	..

- (d) *Lentz Flexner* serum absorbed with two small doses and one very large one of a *W* strain (*Cable*). The agglutinins for *V* and *VZ* are not absorbed. Serum dilution 1 in 10, and for the very large dose, 1 in 100.

Strains against which serum was tested.	Unabsorbed control.	Absorbing doses per c. c. serum.		
		1,000 million.	6,000 million.	480,000 million.
<i>V</i> Lentz Flexner . . .	1,943	1,943	1,943	1,943
<i>VZ</i> Stansfield . . .	1,763	1,763	1,763	1,763
<i>Z</i> Whittington . . .	176	150	150	..
<i>X</i> Hughes . . .	50	35	35	..
<i>WX</i> Mountain 2,464 . . .	150	125	74	..
<i>W</i> Oxford Y . . .	250	74	50	..
„ <i>Cable</i> . . .	69	0	0	..
<i>Y</i> Lentz Y . . .	282	250	250	..

- (e) *Multiple simultaneous absorption* of *Lentz Flexner* serum with moderately large doses (30,000 million of each per c.c. serum) of a *Z* strain (*Lee*), an *X* strain (*Hughes*), and a *W* strain (*Cable*). Serum dilution 1 in 10.

Strains against which serum was tested.	Unabsorbed control.	After absorption.
<i>V</i> Lentz Flexner . . .	2,500	1,656
<i>Z</i> Whittington . . .	165	0
<i>X</i> Hughes . . .	30	0
<i>W</i> Cable . . .	69	0

5. HOMOLOGOUS ABSORPTION OF A *Z* SERUM.

- (a) *Whittington* serum absorbed with its own strain in six doses ranging from very small to moderate. The agglutinins for all races are much reduced, but not in most cases to exhaustion, because a large enough dose was not employed. Serum dilution 1 in 10.

Strains against which serum was tested.	Unabsorbed control.	Absorbing doses per c. c. serum.					
		25 mil-lion.	100 mil-lion.	400 mil-lion.	1,600 mil-lion.	6,400 mil-lion.	18,640 mil-lion.
<i>V</i> Oxford Flexner . . .	68	68	68	63	31	19	0
<i>VZ</i> Stansfield . . .	697	697	607	322	176	155	69
<i>Z</i> Whittington . . .	1,556	1,556	1,556	1,506	613	311	69
„ <i>Lee</i> . . .	1,506	1,506	1,506	1,250	613	301	75
<i>WX</i> Mountain 2,464 . . .	165	165	165	79	50	35	30
<i>W</i> Oxford Y . . .	68	68	65	35	31	25	25
„ <i>Cable</i> . . .	35	35	35	35	33	30	17
„ <i>Huddart</i> . . .	35	35	35	35	33	29	17
<i>Y</i> Lentz Y . . .	165	165	165	155	145	83	65

6. HETEROLOGOUS ABSORPTIONS OF *Z* SERA.

- (a) *Absorption of Whittington* serum with five different doses, small to moderate, of a *V* bacillus (*Oxford Flexner*). The agglutinins for *V* are exhausted, those for *Z* untouched. The titres for *VZ* strains are also untouched. Serum dilution 1 in 10.

Strains against which serum was tested.	Unabsorbed control.	Absorbing doses per c. c. serum.				
		75 million.	300 million.	1,200 million.	4,800 million.	17,200 million.
<i>V</i> Oxford Flexner . . .	75	75	35	33	17	0
„ Lentz Flexner . . .	75	..	75	..	0	0
<i>VZ</i> Stansfield . . .	697	697	697	697	697	697
„ Masom . . .	500	500	500	500	500	500
<i>Z</i> Whittington . . .	1,250	1,250	1,250	1,250	1,250	1,250
„ <i>Lee</i> . . .	849	..	849	..	849	849
„ Allam . . .	1,656	..	1,656	..	1,656	1,656
„ Mountain 2,558 . . .	1,768	1,768	1,768	1,768	1,768	1,768
„ Oxford Y . . .	35	35	35	33	33	33

- (b) *Coles serum* (a year old), absorbed with a single fairly large dose of a pure *V* strain (*Coates*). A repetition of the preceding experiment. The *VZ* agglutinins again behave like *Z* and not like *V*.

Strains against which serum was tested.		Unabsorbed control.	Absorbed with 45,000 million <i>V</i> .
<i>V</i>	Oxford Flexner . . .	84	13
<i>VZ</i>	Stansfield . . .	747	697
"	Waller . . .	872	872
<i>Z</i>	Whittington . . .	3,317	2,937

- (c) *Lee serum* absorbed with two small and two large doses of an *X* strain (*Hughes*) in two experiments, shown separately. The *X* strain is only able to exhaust the agglutinins for *X* and *WX*; the *Z* agglutinin falls only by 25 per cent. Serum dilution 1 in 10 in each experiment.

Strains against which serum was tested.		Unabsorbed control.	Absorbing doses per c. c. serum.	
			1,000 million.	12,500 million.
<i>V</i>	Lentz Flexner . . .	110	74	56
<i>Z</i>	Lee . . .	1,763	1,763	1,250
"	Whittington . . .	2,500	2,200	1,556
<i>X</i>	Hughes . . .	125	15	0
<i>WX</i>	Mountain 2,464 . . .	747	747	79
<i>Y</i>	Lentz <i>Y</i> . . .	65	65	50

Strains against which serum was tested.		Unabsorbed control.	Absorbing doses per c. c. serum.	
			50,000 million.	221,000 million.
<i>V</i>	Lentz Flexner . . .	84	44	44
<i>Z</i>	Lee . . .	1,656	1,250	1,250
"	Whittington . . .	1,656	1,250	1,250
<i>Y</i>	Lentz <i>Y</i> . . .	74	69	69

- (d) *Lee serum* absorbed with a small and a moderately large dose of a *W* strain (*Cable*). The titre for *Z* is wholly unaffected. The secondary agglutinins for *V* and *WX* are moderately reduced. Serum dilution 1 in 10.

Strains against which serum was tested.		Unabsorbed control.	Absorbing doses per c. c. serum.	
			1,000 million.	34,000 million.
<i>V</i>	Lentz Flexner . . .	110	74	50
<i>Z</i>	Lee . . .	1,656	1,656	1,656
"	Whittington . . .	1,763	1,763	1,763
<i>X</i>	Hughes . . .	74	70	70
<i>WX</i>	Mountain 2,464 . . .	727	657	500
<i>W</i>	Cable . . .	26	0	0

- (e) *Multiple simultaneous absorption of Lee serum* with moderately large doses (30,000 million of each, per c.c. serum) of a *V* strain (*Lentz Flexner*), an *X* strain (*Hughes*), and a *W* strain (*Cable*). Serum dilution 1 in 10.

Strains against which serum was tested.		Unabsorbed control.	After absorption.
<i>V</i>	Lentz Flexner . . .	110	0
<i>Z</i>	Whittington . . .	2,500	1,456
<i>X</i>	Hughes . . .	84	0
<i>W</i>	Cable . . .	27	0

7. HOMOLOGOUS ABSORPTIONS OF X SERA.

- (a) *Hughes serum* absorbed with four small to moderate doses of its own bacillus. Primary and secondary agglutinins are alike exhausted, though not to actual extinction. Serum dilution 1 in 10.

Strains against which serum was tested.		Un- absorbed control.	Absorbing doses per c. c. serum.			
			200 million.	800 million.	3,200 million.	12,800 million.
V	Oxford Flexner . . .	653	569	291	50	17
"	Lentz Flexner . . .	803	803	500	69	28
VZ	Stansfield . . .	1,250	1,125	353	65	29
"	Masom . . .	697	697	291	32	15
Z	Whittington . . .	1,557	1,456	1,456	250	69
"	Coles . . .	1,763	1,763	1,456	176	69
"	Lee . . .	1,506	1,506	803	165	68
"	Budden . . .	1,656	1,656	1,250	302	69
"	Mountain 2,558 . . .	1,557	1,557	1,250	302	68
X	Hughes . . .	2,500	2,250	2,250	697	250
"	McCormick . . .	1,125	742	697	311	69
WX	Mountain 2,464 . . .	2,200	2,200	792	282	79
W	Oxford Y . . .	29	29	29	17	15
"	Cable . . .	15	0	0	0	0

- (b) *Reciprocal absorptions* between two X strains, *Hughes* and *McCormick*, demonstrating identity of serological type. Each bacillus is able to exhaust the serum of the other of both primary and secondary agglutinins. The larger of the absorbing doses used was insufficient for complete exhaustion. Serum dilution in each case 1 in 10.

HUGHES SERUM absorbed with small and moderate doses of *McCormick* bacillus.

Strains against which serum was tested.		Unabsorbed control.	Absorbing doses per c. c. serum.	
			1,000 million.	20,000 million.
V	Oxford Flexner . . .	747	322	0
Z	Whittington . . .	1,943	1,456	35
X	Hughes . . .	1,656	1,331	31
"	McCormick . . .	1,100	697	29
WX	Mountain 2,464 . . .	1,763	1,456	33
Y	Lentz Y . . .	166	80	17

MCCORMICK SERUM absorbed with similar doses of *Hughes* bacillus.

Strains against which serum was tested.		Unabsorbed control.	Absorbing doses per c. c. serum.	
			1,000 million.	20,000 million.
V	Oxford Flexner . . .	331	250	22
Z	Whittington . . .	697	322	79
X	Hughes . . .	1,656	1,456	322
"	McCormick . . .	1,250	792	250
WX	Mountain 2,464 . . .	697	697	110
Y	Lentz Y . . .	311	165	59

8. HETEROLOGOUS ABSORPTIONS OF X SERA.

- (a) *Hughes serum* absorbed with small and moderate doses of a V strain (*Oxford Flexner*). The agglutinin for V and VZ is exhausted, that for X is untouched or nearly so. Serum dilution 1 in 10.

Strains against which serum was tested.		Unabsorbed control.	Absorbing doses per c. c. serum.	
			1,000 million.	15,000 million.
V	Oxford Flexner . . .	697	282	0
VZ	Stansfield . . .	792	311	15
Z	Whittington . . .	2,200	1,456	747
X	Hughes . . .	1,948	1,656	1,656
"	McCormick . . .	1,100	1,100	1,100
WX	Mountain 2,464 . . .	1,656	1,656	747
Y	Lentz Y . . .	80	74	17

- (b) *Hughes serum* absorbed with small and moderate doses of a *Z strain* (*Whittington*). The titre for *X strains* is only moderately reduced. The agglutinin for other strains is virtually extinguished. Serum dilution 1 in 10.

Strains against which serum was tested.	Unabsorbed control.	Absorbing doses per c. c. serum.	
		1,000 million.	15,000 million.
V Oxford Flexner .	697	291	30
VZ Stansfield .	792	500	33
Z Whittington .	2,200	1,250	33
X Hughes .	1,948	1,656	1,656
„ McCormick .	1,100	1,100	697
WX Mountain 2,464 .	1,656	1,412	165
Y Lentz Y .	80	80	32

- (c) *Multiple simultaneous absorption of Hughes serum* with small and moderately large doses (1,000 million and 30,000 million of each, per c.c. serum) of a *V strain* (*Lentz Flexner*), a *Z strain* (*Whittington*), and a *W strain* (*Cable*). The titre for *X strains* is only reduced by about one half. Serum dilution 1 in 10.

Strains against which serum was tested.	Unabsorbed control.	Absorbing doses per c. c. serum.	
		1,000 million of each.	30,000 million of each.
V Lentz Flexner .	747	74	0
Z Whittington .	1,556	697	0
X Hughes .	1,557	792	697
„ McCormick .	1,250	792	677
WX Mountain 2,464 .	1,606	747	31

9. HOMOLOGOUS ABSORPTION OF A *W* SERUM.

- (a) *Cable serum* absorbed with five small doses of its own bacillus. The *W* agglutinin is practically exhausted, but the doses were too small to effect more than a partial reduction of the secondary agglutinins for *V* and *Z*. Serum dilution 1 in 10.

Strains against which serum was tested.	Un- absorbed control.	Absorbing doses per c. c. serum.				
		50 million.	200 million.	800 million.	3,200 million.	9,600 million.
V Oxford Flexner .	83	75	65	59	50	50
„ Bennett .	69	..	69	67	67	38
VZ Stansfield .	145	..	125	125	125	80
„ Masom .	72	..	72	72	67	67
Z Whittington .	165	165	142	128	126	125
„ Lee .	125	..	74	74	74	74
„ Coles .	84	..	69	67	67	65
„ Allam .	125	..	83	74	74	69
WX Mountain 2,464 .	331	..	311	150	59	31
W Oxford Y .	917	917	880	618	256	165
„ Cable .	848	848	747	500	155	70
„ Huddart .	747	..	747	331	128	59
Y Lentz Y .	69	..	57	33	30	0

10. HETEROLOGOUS ABSORPTIONS OF *W* SERA.

- (a) *Cable serum* absorbed with a small, a moderate, and a very large dose of a *V strain (Lentz Flexner)*. The titres for *W* and *WX* are only slightly reduced. Serum dilution 1 in 10, and for the very large dose 1 in 100.

Strains against which serum was tested.		Unabsorbed control.	Absorbing doses per c. c. serum.		
			1,800 million.	10,800 million.	432,000 million.
V	Lentz Flexner . . .	74	0	0	..
VZ	Stansfield . . .	165	74	74	..
Z	Whittington . . .	150	150	150	110
WX	Mountain 2,464 . . .	311	291	291	291
W	Oxford Y . . .	848	848	848	697
"	Cable . . .	792	792	697	697
Y	Lentz Y . . .	74	57	57	22

- (b) *Cable serum* absorbed with a small and a moderately large dose of a *VZ strain (Cahourg)*, which reacted fairly well with a *W* serum. The titre for *W* is reduced by about a quarter (Cable) and by 40 per cent. (Oxford Y). Serum dilution 1 in 10.

Strains against which serum was tested.		Unabsorbed control.	Absorbing doses per c. c. serum.	
			1,000 million.	32,000 million.
V	Oxford Flexner . . .	59	0	0
VZ	Cahourg . . .	112	17	0
Z	Whittington . . .	112	104	59
W	Oxford Y . . .	896	530	530
"	Cable . . .	498	360	360

- (c) *Cable serum* absorbed with a small and a large dose of an *X strain (Hughes)*. No effect at all is produced. Serum dilution 1 in 10.

Strains against which serum was tested.		Unabsorbed control.	Absorbing doses per c. c. serum.	
			1,000 million.	50,000 million.
V	Oxford Flexner . . .	69	69	69
Z	Whittington . . .	145	145	145
WX	Mountain 2,464 . . .	291	291	291
W	Oxford Y . . .	792	792	792
"	Cable . . .	697	697	697
Y	Lentz Y . . .	74	74	74

- (d) *Oxford Y serum* absorbed with two doses of a *Z strain (Whittington)*. Only the small agglutinins for *Z* suffer any appreciable reduction. The *W* agglutinin is unaffected. Serum dilution 1 in 10.

Strains against which serum was tested.		Unabsorbed control.	Absorbing doses per c. c. serum.	
			6,250 million	25,000 million.
V	Oxford Flexner . . .	376	331	331
Z	Whittington . . .	36	0	0
"	Lee . . .	29	0	0
W	Oxford Y . . .	3,312	3,312	3,312
"	Cable . . .	5,000	5,000	5,000
"	Huddart . . .	3,212	3,212	3,212

- (e) *Multiple simultaneous absorption of Cable serum with moderately large doses (30,000 million of each per c.c. serum) of a V strain (Lentz Flexner), a Z strain (Lee), and an X strain (Hughes). Serum dilution 1 in 10.*

Strains against which serum was tested.		Unabsorbed control.	After absorption.
V	Lentz Flexner . . .	110	0
Z	Whittington . . .	145	0
X	Hughes . . .	17	0
W	Cable . . .	697	657

11. FURTHER ABSORPTIONS TO ILLUSTRATE THE PROPERTIES OF THE VZ SUB-RACE.

Certain of the absorption protocols detailed above illustrate some of the properties of VZ strains when used for absorbing other sera. Thus 3 a and 3 b show that VZ, as an absorbing strain, behaves as a V rather than as a Z, and the same is shown for secondary agglutinins in 10 b. Again, 6 a and 6 b show that when a Z serum is absorbed with a V strain, the secondary agglutinins for VZ, unlike those for V, are untouched. The following additional experiments were carried out:

- (a) A Z serum (Lee) was absorbed with a VZ strain (Stansfield), in three doses, small to moderately large. The agglutinins for V and VZ are wholly absorbed. Those for Z are only moderately reduced. Serum dilution 1 in 10.

Strains against which serum was tested.		Absorbing doses per c. c. serum.			
		Unabsorbed control.	1,000 million.	10,000 million.	30,000 million.
V	Oxford Flexner . . .	74	31	17	0
"	Lentz Flexner . . .	79	25	0	0
VZ	Stansfield . . .	697	37	0	0
Z	Whittington . . .	2,500	2,500	1,506	1,456
"	Lee . . .	1,763	1,763	1,506	1,250
X	Hughes . . .	79	79	56	37
"	McCormick . . .	69	69	50	35
WX	Mountain 2,464 . . .	747	747	747	747
W	Cable . . .	33	33	33	22
Y	Lentz Y . . .	65	50	29	22

- (b) *Absorption of a VZ serum (Masom) with small and large doses of a V strain (Oxford Flexner), and of a Z strain (Whittington), separately and combined. The V strain is more effectual than the Z in absorbing the agglutinin for VZ; the combination of V and Z is more effectual than either alone. Serum dilutions 1 in 10.*

Strains against which serum was tested.		Absorbed with V alone.		Absorbed with Z alone.		Absorbed with V + Z equal parts.	
		Unabsorbed control.	1,000 million. 90,000 million.	1,000 million. 90,000 million.	1,000 million. 90,000 million.	1,000 million. 90,000 million.	1,000 million. 90,000 million.
V	Oxford Flexner . . .	353	282 0	311 311	311 0		
"	Lentz Flexner . . .	697	353 0		366 31		
"	Bennett . . .	747	500 0		500 33		
VZ	Masom . . .	792	697 176	590 376	564 160		
"	Waller . . .	1,100	747 311	848 697	792 194		
"	Stansfield . . .	1,556	880 500		1,250 250		
Z	Whittington . . .	331	291 125	69 0	125 0		
"	Coles . . .	302	302 138		125 0		
W	Oxford Y . . .	176	165 25		176 20		
"	Cable . . .	35	33 0		33 0		

12. ABSORPTIONS ILLUSTRATING THE PROPERTIES OF THE *WX* SUB-RACE.

- (a) A *Z* serum (*Lee*) absorbed with small and moderate doses of a *WX* strain (*Mountain* 2,464). The *Z* and *VZ* agglutinins are scarcely affected. The titres, originally small, for *X* and *W* strains, are much reduced. The agglutinin for *WX* itself is, naturally, exhausted. Serum dilution 1 in 10.

Strains against which serum was tested.	Unabsorbed control.	Absorbing doses per c. c. serum.	
		1,000 million.	20,000 million.
<i>V</i> Oxford Flexner .	74	74	33
„ Lentz Flexner .	79	56	33
<i>VZ</i> Stansfield .	697	697	697
<i>Z</i> Lee .	1,763	1,557	1,557
<i>X</i> Hughes .	80	50	17
„ McCormick .	69	33	15
<i>WX</i> Mountain 2,464 .	747	87	0
<i>W</i> Cable .	33	15	0
<i>Y</i> Lentz <i>Y</i> .	65	65	29

- (b) A *W* serum (*Cable*) absorbed with small and moderate doses of a *WX* strain (*Mountain* 2,464). Besides absorbing all its own agglutinin the *WX* strain effects a marked reduction in the titre for *W* strains. Serum dilution 1 in 10.

Strains against which serum was tested.	Unabsorbed control.	Absorbing doses per c. c. serum.	
		1,000 million.	20,000 million.
<i>V</i> Oxford Flexner .	74	65	44
<i>Z</i> Whittington .	165	83	80
<i>WX</i> Mountain 2,464 .	331	145	0
<i>W</i> Oxford <i>Y</i> .	848	677	282
„ Cable .	792	697	165
„ Huddart .	848	564	250

- (c) An *X* serum (*McCormick*) absorbed with a small and a moderately large dose of a *WX* strain (*Mountain* 2,464). The small titre for *W* is readily extinguished. The primary *X* agglutinin is reduced by more than one-third. Secondary agglutinins for *V* and *Z* are also much reduced. Serum dilution 1 in 10.

Strains against which serum was tested.	Unabsorbed control.	Absorbing doses per c.c. serum.	
		1,000 million.	30,000 million.
<i>V</i> Oxford Flexner .	331	165	74
<i>Z</i> Whittington .	697	301	145
<i>X</i> Hughes .	1,512	880	848
„ McCormick .	1,250	1,100	803
<i>WX</i> Mountain 2,464 .	742	291	50
<i>W</i> Cable .	29	0	0

- (d) A *WX* serum (*Mountain* 2,464) absorbed with small and moderate doses of a *W* strain (*Cable*). The agglutinins for *W* are completely absorbed,

that for *WX* reduced by 60 per cent., that for *X* scarcely touched. The picture is not that of a homologous absorption. Serum dilution 1 in 10.

Strains against which serum was tested.	Unabsorbed control.	Absorbing doses per c.c. serum.	
		1,000 million.	20,000 million.
V Oxford Flexner .	125	110	36
Z Whittington .	366	311	311
X Hughes .	74	74	74
„ McCormick .	79	69	56
WX Mountain 2,464 .	1,250	697	500
W Oxford Y .	677	183	0
„ Cable .	311	50	0
„ Huddart .	291	31	0

- (e) A *WX* serum (Mountain 2,464) absorbed with small and moderately large doses of an *X* strain (McCormick). The agglutinin for *X* is wholly absorbed, that for *WX* reduced by one-third, that for *W* scarcely touched. The picture is not that of a homologous absorption. Serum dilution 1 in 10.

Strains against which serum was tested.	Unabsorbed control.	Absorbing doses per c.c. serum.	
		1,000 million.	30,000 million.
V Oxford Flexner .	125	68	29
Z Whittington .	331	80	35
X Hughes .	84	0	0
„ McCormick .	79	0	0
WX Mountain 2,464 .	1,250	792	792
W Cable .	311	311	291

- (f) A *WX* serum (Mountain 2,464) absorbed with a small and a large dose of a *W* strain (Cable) and of an *X* strain (Hughes) combined in equal parts. At the same time, the serum was absorbed with its own bacillus. The mixture of *W* and *X* effects practically the same absorption of all agglutinins, primary and secondary as the homologous bacillus.

Strains against which serum was tested.	Un- absorbed control.	Absorbed with its own bacillus.		Absorbed with equal parts of <i>W</i> and <i>X</i> .	
		1,000 million.	26,400 million.	1,000 million of each.	25,000 million of each.
V Oxford Flexner .	155	50	0	69	17
Z Whittington .	352	250	29	176	31
X Hughes .	80	50	0	25	0
WX Mountain 2,464 .	1,412	697	0	500	83
W Cable .	291	145	0	145	0

13. AGGLUTINATION of a *W* strain (Logan), a *WX* strain (Mountain 2,464), and an *X* strain (Toner), with varying mixtures of *W* and *X* sera.

A *W* serum (Logan) and an *X* serum (Toner) were mixed in different proportions and titrated against Logan, Mountain 2,464, and Toner emulsions. The table gives the titres observed. It is seen that the titres for *WX* follow those for *W* and not those for *X*. There is no summation of the effects of the combined sera upon *WX*.

Strains tested.	Logan (<i>W</i>) serum alone.		Toner (<i>X</i>) serum alone.		
	Logan $\frac{2}{3}$, Toner $\frac{1}{3}$.	Logan $\frac{1}{2}$, Toner $\frac{1}{2}$.	Logan $\frac{1}{3}$, Toner $\frac{2}{3}$.		
W Logan .	792	317	317	147	23
WX Mountain 2,464 .	697	280	295	165	147
X Toner .	50	147	165	165	353

14. AGGLUTINATIONS OF EIGHT FURTHER MONOVALENT RABBIT SERA AGAINST THIRTEEN FLEXNER STRAINS. Actual titres.

Three of these sera (Hermann, Clowes, and Howden) were prepared by the late Dr. Gettings with three out of four of his type strains. The remaining sera were of our own preparation; three of them (Toner, Logan, and Ledingham Y) were made from type strains furnished by Captain Murray, R.A.M.C. The Washington Y is the original Hiss and Russell Y from Washington.

	Bacillary strains tested.	SERA									
		V	Z	X	W	Howden.	Washington Y.	Y	Lester Y.	Ledingham Y.	Y
	Hermann.										
V	Lentz Flexner .	2,928	159	472	472	133	332	896	832		
"	Bennett .	2,928	182	472	472	194	313	896	832		
Z	Lee .	254	..	1,016	228	249	188	658	416		
"	Whittington .	118	2,744	944	104	194	143	329	404		
X	Toner .	73	56	3,520	19	66	78	300	440		
"	Hughes .	62	48	1,992	13	59	72	180	249		
WX	Mountain 2,464	267	118	944	3,500	944	358	224	236		
W	Cable .	138	17	14	6,250	1,060	377	127	472		
"	Logan .	164	17	0	7,000	..	358	118	472		
Y	Lentz Y .	416	233	100	466	466	398	880	1,378		
"	Washington Y .	498	208	110	3,500	1,600	848	880	1,888		
"	Ledingham Y .	472	133	110	880	466	796	1,792	2,000		
"	Lester Y .	416	112	106	442	876	452	1,792	1,760		

Note.—The homologous titres are in heavy type. The Clowes serum had a titre for itself of 3,269, the Howden serum one of 1,000, that for Hermann serum was not determined. The potency of the individual sera is seen to vary greatly, and there were probably considerable differences in agglutinability amongst the different bacillary emulsions of the same race. The Washington Y strain, for example, was a highly agglutinable one.

On comparing this table with the one given in Appendix: 2, it will be seen that the properties of the sera of the different races are similar in the two sets of agglutinations

15. CROSS ABSORPTIONS BETWEEN DIFFERENT Y STRAINS.

- (a) *Lentz Y* serum absorbed with small and large doses of the *Lister Y* strain. The agglutinins for all strains are very greatly reduced, but the primary agglutinin for *Lentz Y* itself is the least affected, 14 per cent. remaining. The picture on the whole is that of a homologous absorption. Serum dilution 1 in 10.

Strains against which serum was tested.		Unabsorbed control.	Absorbing doses per c.c. serum.	
			1,120 million.	73,920 million.
V	Oxford Flexner .	424	352	18
Z	Whittington .	377	203	0
W	Cable .	101	94	13
Y	<i>Lentz Y</i> .	745	563	106
„	<i>Lister Y</i> .	570	450	12
„	Washington Y .	2,066	1,593	72

- (b) *Lister Y* serum absorbed with small and large doses of the *Lentz Y* strain. In this experiment, the reciprocal of the preceding, absorption is far less complete; more than 50 per cent. of the specific *Lister Y* agglutinin remains unabsorbed.

Strains against which serum was tested.		Unabsorbed control.	Absorbing dose per c.c. serum.	
			1,256 million.	77,244 million.
V	Oxford Flexner .	101	76	49
Z	Whittington .	165	106	94
W	Cable .	101	88	53
Y	<i>Lentz Y</i> .	165	51	0
„	<i>Lister Y</i> .	1,446	1,126	796
„	Washington Y .	452	226	113

- (c) *Lentz Y* serum absorbed with a single large dose of the *Washington Y* strain. The effect is practically that of a homologous absorption. The titre which suffers least is that for *Lentz Y* itself, only three-quarters of the primary agglutinin being absorbed. Serum dilution 1 in 10.

Strains against which serum was tested.		Unabsorbed control.	Absorbed with 67,000 million per c.c. serum.
V	Oxford Flexner .	406	83
Z	Whittington .	358	48
W	Cable .	226	0
Y	<i>Lentz Y</i> .	1,811	481
„	<i>Lister Y</i> .	1,331	176
„	Washington Y .	1,491	60

- (d) *Washington Y* serum absorbed with a small and a moderately large dose of the *Lentz Y* strain. The reciprocal of the preceding experiment. Here the *Lentz Y* bacillus effects almost as good an absorption as did the *Washington Y* in 15 (c). Serum dilution 1 in 10.

Strains against which serum was tested.		Unabsorbed control.	Absorbing doses per c.c. serum.	
			1,110 million.	31,800 million.
V	Oxford Flexner .	94	47	12
Z	Whittington .	88	88	20
X	Hughes .	18	18	18
W	Cable .	226	186	87
Y	<i>Lentz Y</i> .	310	164	12
„	<i>Lister Y</i> .	358	211	77
„	Washington Y .	452	398	120

Note.—The low titres in this experiment are due to the fact that the absorbing mixtures and control were inadvertently left all night at 55° C., but the control suffered with the others, so that the figures are still comparable.

16. HETEROLOGOUS ABSORPTIONS OF Y SERA.

- (a) *Lentz Y serum* absorbed with a *V strain (Lentz Flexner)* and with a *W strain (Cable)* in small and moderately large doses, separately and combined. The *V strain* effects a much greater reduction in the agglutinin than the *W strain*, but even the large dose of *V* leaves 42 per cent. of the *Y agglutinin* for *Lentz Flexner* and 63 per cent. of that for the *Lister Y*. The absorption with *V* and *W* combined does little more than the *V strain* alone. Serum dilutions 1 in 10.

Strains against which serum was tested.		Unabsorbed control.	Absorbed with <i>V</i> alone.		Absorbed with <i>W</i> alone.		Absorbed with <i>V + W</i> (equal parts).	
			1,000 million.	32,000 million.	1,000 million.	32,000 million.	900 million of each.	32,000 million of each.
<i>V</i>	Oxford Flexner	311	165	0	301	301	176	0
<i>Z</i>	Whittington	282	282	69	282	176	282	50
<i>W</i>	Cable	29	17	0	0	0	0	0
<i>Y</i>	Lentz Y	1,656	1,656	697	1,506	1,456	1,656	792
„	Lister Y	1,100	792	697	792	747	792	697

- (b) *Lentz Y serum* simultaneously absorbed with small and moderately large doses of *V, Z, X,* and *W strains* in equal parts (*V*=Oxford Flexner, *Z*=Whittington, *X*=Hughes, *W*=Cable). The titre for *Lentz Y* and *Lister Y* suffers most, but 22 per cent. and 18 per cent. of the *Y agglutinin* for these strains remains even after the large combined dose. The picture is almost, but not quite, that of a homologous absorption. Serum dilution 1 in 10.

Strains against which serum was tested.		Unabsorbed control.	Absorbing doses per c.c. serum.	
			800 million of each = 3,200 million.	32,000 million of each = 128,000 million.
<i>V</i>	Oxford Flexner	796	700	23
<i>Z</i>	Whittington	504	377	12
<i>W</i>	Cable	188	155	0
<i>Y</i>	Lentz Y	809	706	176
„	Lister Y	452	376	82
„	Washington Y	1,695	1,510	188

- (c) *Reciprocal absorptions between the Lister Y and a V strain (Lentz Flexner)* in small and moderately large doses. Sixty per cent. of the specific *Lister Y agglutinin* is absorbed by the *V strain*, but in the converse experiment the *V titre* is untouched by absorption with *Lister Y*. Serum dilutions 1 in 10.

Lister Y serum absorbed with Lentz Flexner Bacillus.

Strains against which serum was tested.		Unabsorbed control.	Absorbing doses per c.c. serum.	
			1,200 million.	25,000 million.
<i>V</i>	Lentz Flexner	284	84	0
<i>Z</i>	Whittington	281	105	84
<i>X</i>	Hughes	18	18	18
<i>W</i>	Cable	178	82	64
<i>Y</i>	Lister Y	2,253	1,265	905
„	Lentz Y	281	124	54

Lentz Flexner Serum absorbed with Lister Y Bacillus.

Strains against which serum was tested.		Unabsorbed control.	Absorbing doses per c.c. serum.	
			1,200 million.	25,000 million.
<i>V</i>	Lentz Flexner	1,696	1,696	1,696
<i>Z</i>	Whittington	188	47	44
<i>X</i>	Hughes	41	41	38
<i>W</i>	Cable	77	29	14
<i>Y</i>	Lister Y	563	176	15
„	Lentz Y	188	100	26

- (d) *Reciprocal absorptions between the Lister Y and a Z strain (Whittington)* in small and moderately large doses. The Z bacillus effects a reduction of only 23 per cent. in the specific Y agglutinin. The Y bacillus effects a 50 per cent. reduction in the specific Z agglutinin. Serum dilutions 1 in 10.

Lister Y Serum absorbed with Whittington Bacillus.

Strains against which serum was tested.	Unabsorbed control.	Absorbing doses per c.c. serum.	
		1,110 million.	25,000 million.
V Oxford Flexner .	125	120	100
Z Whittington .	179	63	13
X Hughes .	106	106	106
W Cable .	89	25	10
Y Lister Y .	1,696	1,696	1,318
„ Lentz Y .	212	212	155

Whittington Serum absorbed with Lister Y Bacillus.

Strains against which serum was tested.	Unabsorbed control.	Absorbing doses per c.c. serum.	
		1,110 million.	25,000 million.
V Oxford Flexner .	77	56	25
Z Whittington .	1,126	1,126	563
X Hughes .	124	88	50
W Cable .	23	18	0
Y Lister Y .	796	397	12
„ Lentz Y .	106	94	0

- (e) *Reciprocal absorptions between the Lister Y and a W strain (Cable)* in small and moderately large doses. Only about one-third of the specific Y agglutinin is absorbed by the W strain. Not quite half the specific W agglutinin is absorbed by the Y strain. Serum dilutions 1 in 10.

Lister Y Serum absorbed with Cable Bacillus.

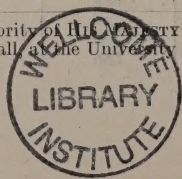
Strains against which serum was tested.	Unabsorbed control.	Absorbing doses per c.c. serum.	
		1,180 million.	25,000 million.
V Lentz Flexner .	155	84	84
Z Whittington .	155	144	144
X Hughes .	70	70	70
W Cable .	77	0	0
Y Lister Y .	716	452	452
„ Lentz Y .	117	82	82

Cable Serum absorbed with Lister Y Bacillus.

Strains against which serum was tested.	Unabsorbed control.	Absorbing doses per c.c. serum.	
		1,200 million.	25,000 million.
V Lentz Flexner .	310	53	23
Z Whittington .	12	0	0
X Hughes .	0	0	0
W Cable .	620	332	332
Y Lister Y .	101	12	0
„ Lentz Y .	46	10	0

- (f) *Lister Y serum simultaneously absorbed with V, Z, X, and W strains*, 10,000 million each of Oxford Flexner, Whittington, and Cable, and 7,500 million of Hughes, per c.c. serum. Only the single dose was used. Half the agglutinin for Lister Y was removed, and about three-quarters of that for Lentz Y and Washington Y. Serum dilution 1 in 10.

Strains against which serum was tested.	Unabsorbed control.	After absorption.
Y Lister Y	796	398
„ Lentz Y	212	56
„ Washington Y	176	41



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